Synthesis and Antitumor Activity of New Glycosides of Epipodophyllotoxin, Analogues of Etoposide, and NK 611

Laurent Daley,[†] Yves Guminski,[‡] Pierre Demerseman,[†] Anna Kruczynski,[§] Chantal Etiévant,[‡] Thierry Imbert,[‡] Bridget T. Hill,[§] and Claude Monneret*[,†]

UMR 176 CNRS/Institut Curie, Section Recherche, 26 rue d'Ulm, F-75248 Paris Cedex 05, France, and Pierre Fabre Médicament, C.R.P.F., 17 Avenue Jean-Moulin, F-81106 Castres, France

Received February 2, 1998

A series of 3-amino- and 3-alkylamino-2-deoxy-β-D-*ribo*- and β-D-*arabino*-glycosides of 4′-demethylepipodophyllotoxin have been synthesized by means of an improved trimethylsilyl-iodide procedure for the podophyllotoxin-4′-demethylepipodophyllotoxin conversion, an efficient and high yielding synthesis of silyl glycoside donors of 3-azido-2,3-dideoxy-β-D-*ribo*- and β-D-*arabino*-hexopyranosides and stereoselective glycosylations. In vitro evaluation of cytotoxic effects against murine L1210 leukemia critically demonstrates the essential role played by a 4,6-acetal for biological activity. Among the most cytotoxic compounds, 3-amino-2,3-dideoxy- and 3-*N*,*N*-(dimethylamino)-2,3-dideoxy etoposide analogues, **17** and **27**−**29** are at least as potent as etoposide on the in vivo P388 (iv/ip) murine leukemia models. However, surprisingly enough, none of these compounds inhibits the human DNA topoisomerases I or II or binds to tubulin to prevent its polymerization and microtubule assembly. Therefore, their mechanism of action remains to be cleared up.

Introduction

Following the discovery of the antitumor activity of the natural lignan, podophyllotoxin,[1] clinical trials were undertaken in the 1950s.[2] However, these trials were stopped because of excessive toxicity and lack of clinical response. Nevertheless, as a result of a general program in attempts to find a less toxic podophyllotoxin analogue and/or derivative, 4′-demethylpodophyllotoxin 4-(4,6-*O*-ethylidene)-β-D-glucopyranoside (or etoposide or VP-16) and the corresponding 4,6-*O*-thenylidene (or teniposide or VM-26) emerged as the most interesting glucosides in the 1970s.[3]

Etoposide is now widely used in clinical cancer chemotherapy, alone or in association, for the treatment of testicular carcinomas and small cell lung cancer[4] but its current therapeutic use is somewhat limited by myelosuppression, particularly neutropenia.[5] Unlike podophyllotoxin, etoposide and teniposide do not inhibit tubuline polymerization but induce dose-dependent DNA-strand breakage associated with inhibition of DNA-topoisomerase II.[6] Therefore, the researchers' efforts in this area were directed toward modification of epipodophyllotoxin glucosides as potent topoisomerase II inhibitors by synthesizing amino glycoside,[7] thio glycoside,[8] and *N*- or *C*-glycoside analogues.[9,10]

Among all these new glycosides, NK 611,[7b] which differs from etoposide in the replacement of the glucose moiety with the 2-*N*,*N*-dimethylglucosamine residue, has raised clinical trials for the past few years. Like etoposide, NK 611 inhibits topoisomerase II activity but is, advantageously, 120-fold more soluble in water than etoposide. Following preliminar clinical information,[11] further development of this drug in phase II trials is soon expected.

In this report, we describe the synthesis of new glycosides of epipodophyllotoxin and the in vivo structure−activity relationships of this new series.

Although less active than NK 611, 3-amino-β-D-glucoside analogues were nevertheless found[7a] to have higher activity than etoposide against murine L1210 leukemia cell lines. In view of these results, we were interested in further modifications of the sugar moiety. Therefore, we turned our attention toward 3-amino-2,3-dideoxy sugars, closely related to the corresponding 3-amino-2,3,6-trideoxy-L-hexoses[12] occurring in natural antibiotics belonging to the anthracycline family[13] and to the vancomycin group.[14] Lacking the OH group at C-2, such 3-amino-2,3-dideoxy D-sugars were revealed to possess an interesting balance between hydrophilicity and lipophilicity with respect to intracellular penetration while keeping the faculty to afford water-soluble salts such as hydrochlorides. This feature has already been used to prepare potent antitumor nitrosourea-sugar derivatives such as ecomustine,[15] a compound endowed of potent antitumor activity and, above all, highly active against colon-38 carcinoma.[16]

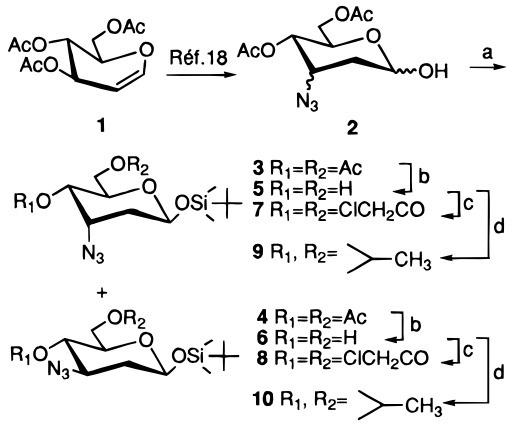
Chemistry

Ten years ago, some of us reported[17] a highly efficient synthesis of 3-amino-2,3,6-trideoxy-L-hexoses starting from 3,4-di-*O*-acetyl-L-rhamnal (or 3,4-di-*O*-acetyl-6-deoxy-L-glucal) and, very recently,[18] this synthesis was extended to the 3,4,6-tri-*O*-acetyl-D-glucal **1**. Based upon Michael addition of NaN_3 to the corresponding hex-2-enopyranose, this methodology straightly afforded 3-azido-2,3-dideoxy precursors **2** in a one-pot reaction (Scheme 1). The problem was the control of the stereochemistry at the anomeric position in order to separate the *arabino* and the *ribo* isomers. This could be

[†] UMR 176 CNRS/Institut Curie, Section Recherche, Paris.
[‡] Pierre Fabre Médicament C.R.P.F., Division de Chimie Médicinale.
[§] Pierre Fabre Médicament C.R.P.F., Division Cancérologie Expérimentale.

10.1021/jm9800752 CCC: $15.00
Published on Web 10/17/1998

Scheme 1[a]



[a] Reagents: (a) TBDMSCl, imidazol; (b) MeONa/MeOH; (c) $ClCH_2COCl$, pyridine; (d) $CH_3CO(OEt)_2$, APTS.

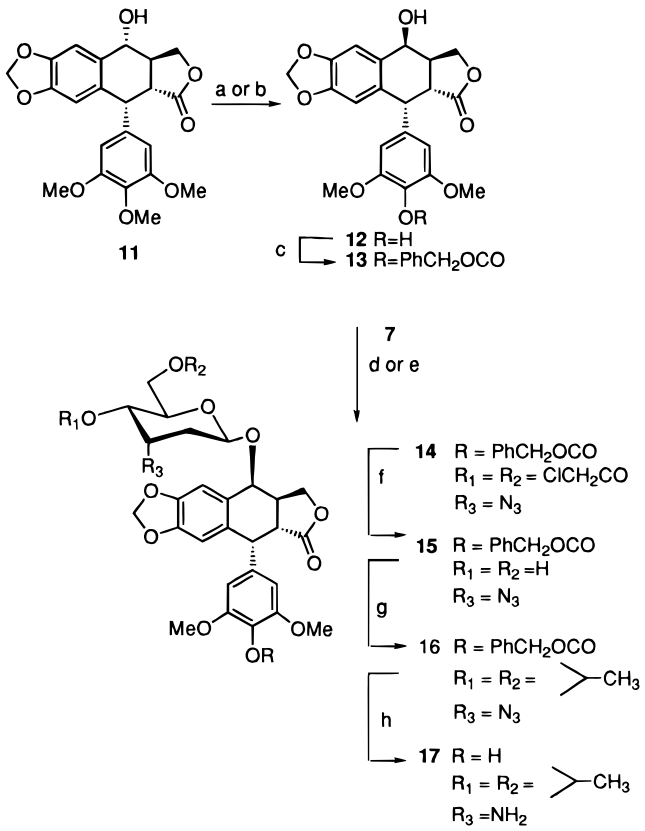
achieved by using pivaloyl chloride and pyridine, since only β-anomers were observed under these conditions resulting from a kinetically preferred β-alkylation.[19] In a next investigation, we decided to look toward the formation of 1-*O*-*tert*-butyldimethylsilyl glycosides which are known as excellent glycosyl donors for the synthesis of 2-deoxy glycosides.[20,21] Moreover usually silylation of the free anomeric OH group in DMF/imidazole leads predominantly, or stereoselectively, to the β-anomers in the range of 80−95%.

Indeed treatment of the crude azido sugar mixture **2** with *tert*-butyldimethylsilyl chloride in the presence of imidazole afforded the azido derivatives of β-D-*ribo* and β-D-*arabino* configuration, **3** and **4**, respectively, in 75% overall yield and 1:2 ratio. Subsequent transesterification (NaOMe−MeOH) led to the corresponding 4,6-diols **5** and **6** which were immediatly protected as their chloroacetyl esters **7** and **8**.[22] Alternatively, these azido sugars **5** and **6** were converted into their 4,6-*O*-ethylidene acetals **9** and **10**, with the acetal group present in etoposide itself, by treatment with 1,1-diethoxyethane/TsOH.

On the other hand, classical conversion of podophyllotoxin **11** into 4′-demethyl-epipodophyllotoxin simultaneously and selectively deprotected at the 4′-position, such as **12**, involved treatment of **11** with HBr in 1,2-dichloroethane−ether (10:1) at 0 °C followed by hydrolysis ($BaCO_3$ in H_2O).[23] A slightly modified version was next proposed by Lee et al.[24] with HBr gas in CH_2Cl_2 but in an overall yield not exceeding 52%. Considering these literature data (low yield and lack of reproductive yield on higher scale), we decided to explore the possibility of using trimethylsilyl iodide (TMSI) instead of HBr. Indeed, it has been shown that TMSI is able not only to selectively cleave[25] the 2-OMe ether in the 2,3-dimethoxytoluene but also to convert a hydroxyl group into the corresponding iodo derivative with inversion of configuration.[26−29] Moreover, it should be noticed that the potential usefulness of TMSI for the two reactions aforementioned has been exemplified in one report[30] on the synthesis of structural analogues of epipodophyllotoxin, although not in a sequential application.

Therefore, podophyllotoxin **11** was treated with TMSI and barium carbonate to afford **12** which was subsequently protected at the 4′-position as its benzyl carbonate, leading to **13** (≈70% overall yield)[31] (Scheme 2).

Scheme 2[a]

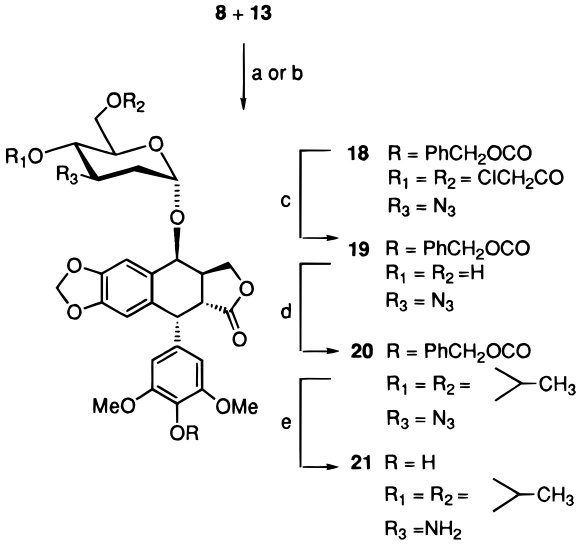


[a] Reagents: (a) HBr then $BaCO_3$; (b) TMSI then $BaCO_3$; (c) $PhCH_2OCOCl$, Et_3N; (d) BF_3-Et_2O; (e) TMSOTf; (f) Amberlite OH^-; (g) $CH_3CH(OEt)_2$, TsOH; (h) H_2, Pd/C.

The first coupling reaction of **13** was undertaken with the glycosyl donor of D-*ribo* configuration **7**, in the presence of trimethylsilyltriflate (TMSOTf). The β-glycoside **14** was selectively isolated in 50% yield and then deprotected (Amberlite OH^-) on the sugar moiety to give **15** in 97% yield. This was treated with 1,1-diethoxyethane in the presence of *p*-TsOH to afford (66%) the corresponding 4,6-*O*-ethylidene derivative **16**. Alternatively, **16** was also prepared in 36% yield by glycosylation of **13** with the glycosyl donor having the 4,6-acetal group already present, i.e., **9**, in the presence of TMSOTf. However, in this case, better yield was obtained when boron trifluoride etherate was used (47%) instead of TMSOTf. The β-*ribo*-glycoside **16** was subsequently converted into **17** (H_2, 10% Pd/C, 88% yield).

The highly stereoselective formation of the β-glycoside may be explained in terms of kinetic product resulting from the attack of the intermediate enoxonium ion on the opposite side of the azido group leading to antiparrallel dipoles at C_1-C_3. This hypothesis seems to be in agreement with the next result obtained with the azido sugar of D-*arabino* configuration **8**. In this case, condensation with epipodophyllotoxin **13**, under the same conditions as above, resulted in the exclusive formation of the α-glycoside **18** in 65% yield (Scheme 3). In this latter case, the higher yield and stereoselectivity can be seen as the result of both kinetic and thermodynamic controls. This α-*arabino*-glycoside **18** was then deprotected (**19**) (Amberlite OH^-) and successively converted

Scheme 3[a]



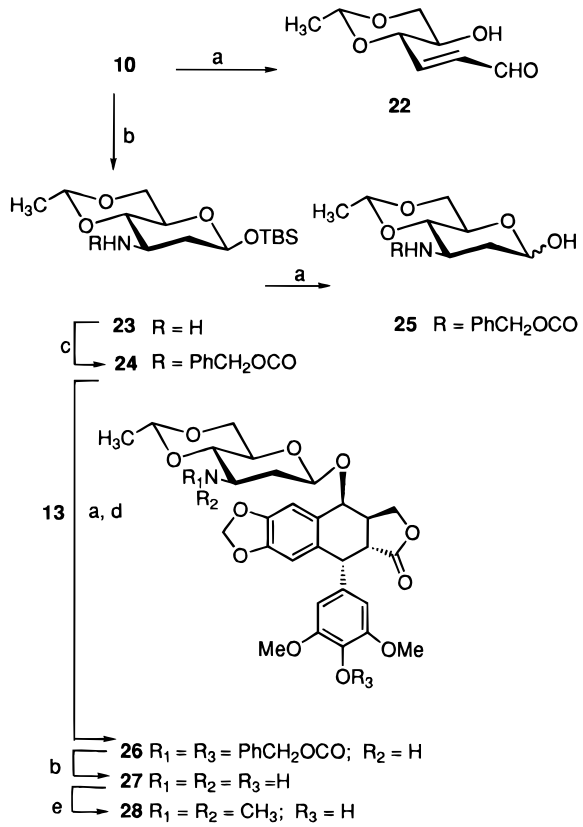
[a] Reagents: (a) BF_3Et_2O; (b) TMSOTf; (c) Amberlite OH^-; (d) $CH_3CH(OEt)_2$, TsOH; (e) H_2, Pd/C.

into **20** (1,1-diethoxyethane, *p*-TsOH, 90%) and **21** (H_2, 10% Pd/C, 75%).

The remaining problem was to prepare the β-glycosides of *arabino* configuration, since it has been reported that only the β-glycosides of epipodophyllotoxin were endowed with antitumor properties.[32] One way to overcome this difficulty could be found in a glycosidation reaction type Kuhn,[33] which involved a reverse addition mechanism by formation of a benzylic cation on the aglycon moiety and attack from a 1-β-OH sugar. At first compound **10** was treated with Bu_4NF in THF to deliver the reduced analogue by cleavage of the O−Si bond. This led to the *E*-unsaturated compound **22** proceeding from a retro-Michaël reaction with departure of the azido group. To avoid this problem, the azido group was reduced prior to the desilylation step and the amino derivative **23** protected as its benzyloxycarbonyl derivative **24** (70% overall yield). In this case, desilylation of **24** gave the expected reduced analogue **25** but as a mixture of α- and β-anomers in a 1:1 ratio after extraction, as shown by ^1H NMR spectrum. One way of avoiding this epimerization, which probably occurs during the workup, could be through the formation of free sugar in situ, followed by immediate glycosidation without any purification. Consequently, we decided to undertake a "one-pot" reaction involving successive additions to **24** of Bu_4NF (1.2 equiv), epipodophyllotoxin derivative **13** (1.1 equiv), and lastly, $BF_3{\cdot}Et_2O$ catalyst (15 equiv). Such a hypothesis proved to be right, since this exclusively afforded the β-glycoside **26** in 54% yield. The next transformation involved reductive hydrogenolysis to afford **27** (90%).

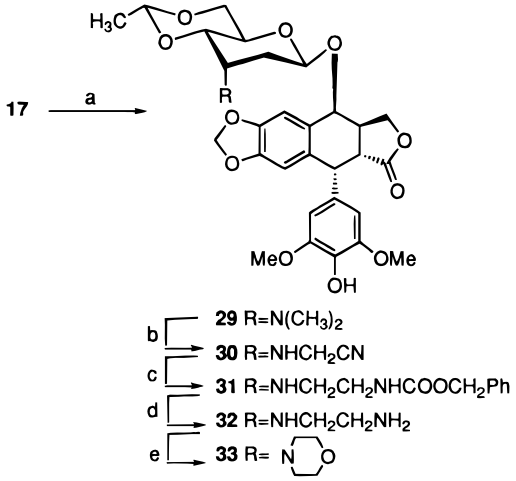
To compare the activity of new glucosides with NK 611, our next objective was to prepare the 3-dimethylamino derivatives, namely **28** and **29**, by reductive alkylation (HCHO, $NaBH_3CN$) of **17** and **27**, respectively (Schemes 4 and 5). For their part, *N*-alkyl derivatives such as *N*-cyanoethyl, *N*-aminoethyl, and *N*-morpholino residues, **30**, **32**, and **33**, were obtained (Scheme 5) by condensation of the amino-glycoside of *ribo* configuration **17** with the appropriate alkyl iodide in the presence of Et_3N. In the case of **32**, the intermediate **31** was deprotected by hydrogenolysis in the presence of 10% Pd/C.

Scheme 4[a]



[a] Reagents: (a) Bu_4NF/THF; (b) H_2, Pd/C; (c) $PhCH_2OCOCl$, Et_3N; (d) BF_3/Et_2O; (e) HCHO, $NaBH_3CN$.

Scheme 5[a]

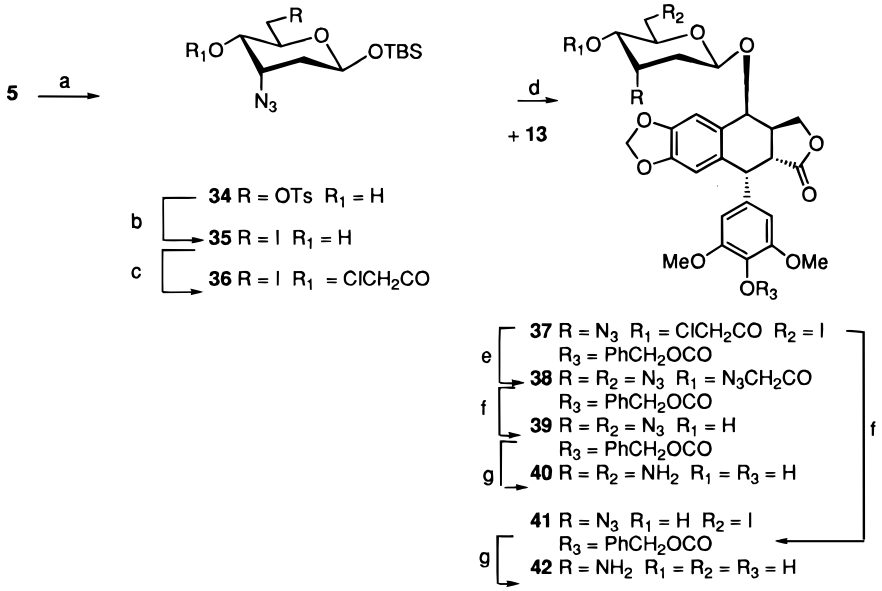


[a] Reagents: (a) HCHO, $NaBH_3CN$; (b) ICH_2CN, Et_3N; (c) ICH_2CH_2NHZ, Et_3N; (d) H_2, Pd/C; (e) $(ICH_2CH_2)_2O$, Et_3N.

To ascertain whether the presence of an acetal group at C-4−C-6 was a requisite condition for biological activity as observed in the glycoside series, the azido sugar **5** was converted (Scheme 6) into the key intermediate 6-iodo-6-deoxy monosaccharide **36** via selective tosylation at C-6, replacement of the tosyloxy group by iodine, and then chloroacetylation (**5** → **34** → **35** → **36**) in 50% overall yield.

This iodo sugar **36** was condensed with **13** to cleanly afford (48%) the β-glycoside **37**. Introduction of an azido

Scheme 6[a]



[a] Reagents: (a) TsCl, pyridine; (b) NaI, acetone; (c) $ClCH_2COCl$, pyridine; (d) BF_3/Et_2O; (e) NaN_3; (f) Amberlite OH^-; (g) H_2, Pd/C.

Table 1. In Vitro Cytotoxic Activity

compd	L1210 IC_{50} (μM)[a]
17	0.23
21	1.3
27	0.07
28	0.11
29	0.09
30	2.0
32	1.7
33	>1.0
40	0.21
42	12
NK 611	0.33
etoposide	0.20

[a] IC_{50}: concentration of drug required to reduce cell growth to 50% of that obtained with control cell.

Table 2. In Vitro Activity on Topoisomerase and Tubulin

compd	topoisomerase II EC_{50} (μM)[a]	TPI IC_{50} (μM)[b]
17	>100	>10
21	>100	>50
27	>100	>50
28	>100	>50
29	>100	>50
30	>1	>100
32	>100	>10
33	>1	NT
40	>100	11
42	5.6	>10
etoposide	56	NT
NK 611	56	NT
podophyllotoxin	NT[c]	3
4′-DMEP	NT	13

[a] EC_{50}: concentration at which any inhibitory effect could be detected. The EC_{50} values reported here represent the mean of two separate experiments. [b] IC_{50}: concentration leading to 50% tubulin polymerization. The IC_{50} values reported here were determined from five independent experiments. [c] Not tested.

group by reacting **37** with NaN_3 led to the triazido glycoside **38** (90%) resulting from the simultaneous Cl substitution as present in the chloroacetyl group. 3,6-Diamino glycoside **40** was obtained by treatment of **38** with Amberlite (IRA 410 OH^-) and hydrogenolysis (89% overall yield) of intermediate **39**. Alternatively, **37** was subsequently transformed by Amberlite treatment into **41**, and hydrogenolysis of **41** (36% overall yield) led to the corresponding 3-amino-6-deoxy glycoside **42**.

Results and Discussion

In vitro cytotoxic activity results assessed with the L1210 murine leukemia model are shown in Table 1. β-glycosides of 4′-demethylepipodophyllotoxin having a 3-amino-2,3-dideoxy or a 3-(dimethylamino)-2,3-dideoxy sugar moiety along with a 4,6-*O*-ethylidene acetal, i.e., **17**, **27**, **28**, and **29** showed the same order of magnitude in terms of cytotoxic potency, compared to etoposide or NK 611 reference compounds. The α-glycosyl derivative **21** as well as the aminoalkyl derivatives **30**−**33** were less cytotoxic. Among the two compounds which did not feature an ethylidene group, the 3′-amino-2′,3′,6′-trideoxy glycoside **42** was nearly inactive whereas, for a priori unexplainable reason, the 3′,6′-diamino-2′,3′,6′-trideoxy glycoside **40** was endowed with potent cytotoxic activity. Indeed this latter result is in contrast with the accepted results within the etoposide or NK 611 series, where an ethylidene acetal was proved to be an important structural requirement to enhance cytotoxic properties.[7a,b]

To investigate the mechanism of action of these new glycosides, inhibition of topoisomerases I and II and tubulin polymerization inhibition experiments were carried out, and results are shown in Table 2. None of the synthesized compounds interfered with topoisomerase II, considered as a classical target for epipodophyllotoxin derivatives,[34] up to a 10^{-4} M concentration. Only one compound (**42**), without the ethylidene acetal moiety, consistently inhibited the capacity of topoisomerase II (EC_{50} = 5.6 μM) to cleave double-stranded kDNA with a 10-fold higher activity than NK 611 and etoposide (IC_{50} = 56 μM). As compound **42** is not cytotoxic and constitutes a potent topoisomerase II inhibitor, correlatively with **27**, **28**, **17**, **29**, and **40** which are cytotoxic and not topoisomerase II inhibitors, the

Table 3. In Vivo P388 Murine Leukemia (iv/ip)

compd	water solubility (mg/mL)	ED_{50} (mg/kg)	T/C max (%) [dose (mg/kg)]
17 (α-NH_2)	11	5	300 [160]
21 (β-NH_2)	10	inact. (40)	
27 (β-NH_2)	9	1.7	193 [40]
28 (β-NMe_2)	24	1.7	243 [40]
29 (α-NMe_2)	26	5	257 [40]
30 (α-$NHCH_2CN$)	ins.	20	186 [160]
32 (α-$NH(CH_2)_2NH_2$)	40	inact. (40)	
33 (α-*N*-morpholino)	ins.	inact. (40)	
40	42	inact. (40)	
42	13	inact. (40)	
NK 611	15	0.9	228 [20] 100 [40]
etoposide	0.01	5	242 [160]

problem of direct correlation between inhibition of this enzyme and cytotoxic activity is posed in this new series.

Though tubulin polymerization inhibition (TPI) was lost in the epipodophyllotoxin series relative to podophyllotoxin, as already known, this inhibition test was also performed. Interestingly, compound **40** showed a modest but consistent inhibition of TPI ($IC_{50} = 11\ \mu M$) closely related to that of 4′-demethyl-epipodophyllotoxin (4′-DMEP) with an $IC_{50} = 13\ \mu M$. The 4′-DMEP proved to be slightly less cytotoxic than podophyllotoxin (internal results). Hence, the cytotoxic activity of **40** ($IC_{50} = 0.21\ \mu M$) could hardly be attributed to its TPI mechanism.

In vivo antitumor activity displayed by the studied compounds against the P388 murine leukemia model (iv/ip) is recorded in Table 3. The compounds of the *arabino* series **27** and **28**, with a 3″-β-amino group in equatorial position relative to the pyran ring, appeared nearly as potent as NK 611, with an ED_{50} equal to 1.7 mg/kg versus 0.9 mg/kg for NK 611. They exhibited a relatively greater potency than the *ribo* analogues **17** and **29**, with a 3″-α amino group in an axial position relative to the pyran ring. Compounds **17** and **29**, with an $ED_{50} = 5$ mg/kg, were shown to be as potent as etoposide. No difference was shown between primary and tertiary amine substitution, **27** versus **28** and **17** versus **29**. Moreover, bulky axial amino groups, as in **30**, **32**, and **33**, reduced (**30**) or totally abolished activity (**32**, **33**). These results indicated the unfavorable occupancy of the α-side of the glucoside moiety. It is noteworthy that compounds **27**, **28**, **17**, and **29** induced increased the life span of P388 leukemia-bearing mice with a T/C ratios ranging from 193 to 300%; these values are identical or higher than that for NK 611 or etoposide. Consequently, compounds **27**, **28**, and **29** could be administrated up to 40 mg/kg and **17** up to 160 mg/kg to obtain the greatest increase of lifespan, in comparison with NK 611. So, these compounds appeared less toxic than NK 611 and they would afford a better putative flexibility in the dose schedule than NK 611, with which activity was lost at 40 mg/kg. Water solubility made these compounds attractive, the values recorded in Table 3 proved to be sufficiently adequate to overcome administration problems.

In conclusion, new water-soluble epipodophyllotoxin derivatives were synthesized and evaluated in vitro and in vivo. In vitro L1210 cytotoxic assay demonstrated an equipotent activity for five compounds compared with reference compounds (i.e., **27**, **28**, **17**, **29**, **40**). No in vitro activity was shown against topoisomerase I, II, or tubulin for compounds which displayed in vivo activity. Potent in vivo activity was displayed for two compounds, **27** and **28**, on the P388 (iv/ip) murine leukemia model, relative to the reference compounds, while two others, **17** and **29**, proved as potent as etoposide. Though 10 times more potent than the reference compounds against topoisomerase II enzyme, compound **42** did not show any in vivo antitumor activity in the P388 test.

Further studies are currently underway to assess the potential activity of these analogues against DNA as minor grove binders or against putative target enzymes and to enlighten their mechanism of action.

Experimental Section

Chemical Methods. General Procedure. Melting points were determined using an Electrothermal apparatus and are uncorrected. UV spectra were determined on a Varian-Cary/3E spectrophotometer. IR spectra were obtained with a Perkin-Elmer 1710 spectrophotometer. ^1H NMR spectra were recorded in the given solvent with a Bruker AC-250 spectrometer. Chemical shifts are reported as δ values in parts per million. The splitting pattern abbreviations are as follows: s = singlet, d = doublet, dd = double doublet, dt = double triplet, t = triplet, br = broad, m = multiplet. Chemical ionization (CI) mass spectra were recorded on a Nermag R 10,10C spectrometer. Elemental analyses, performed by the Service de Microanalyse du CNRS (Vernaison-Lyon, France), were within 0.4% of the theoretical values calculated for C, H, and N. The thin-layer chromatographic analyses were performed using precoated silica gel ($60F_{254}$) plates, and the spots were examined with UV light and phosphomolybdic acid spray. Column chromatography was carried out on Merck silica gel (230−240 mesh). Extraction in usual manner refers to washing the organic layer with water, drying it over $MgSO_4$, and evaporating the solvent under reduced pressure.

***tert*-Butyldimethylsilyl 3-Azido-4,6-di-*O*-acetyl-2,3-dideoxy-β-D-*ribo* (3) and β-D-*arabino* (4) Hexopyranosides.** To a cooled solution (0 °C) of the crude mixture **2**[18] (16.1 g, 58 mmol) in anhydrous dichloromethane (200 mL) were added imidazole (6 g, 87.8 mmol) and *tert*-butyldimethylsilyl chloride (13.24 g, 87.8 mmol). The reaction mixture was stirred at the same temperature for 0.5 h and then allowed to reach room temperature with further stirring for 18 h. It was poured into H_2O (500 mL) and extracted with CH_2Cl_2 (300 mL). The organic layer was separated and dried over anhydrous $MgSO_4$. Flash chromatography (9:1 cyclohexane/EtOAc) of the residue (18.7 g) successively led to **3** (4.5 g, 20%), to a mixture of **3** and **4** (2.7 g, 12%), and then to **4** (9.5 g, 43%).

Compound **3**, syrup: R_f 0.52 (4:1 cyclohexane/EtOAc); $[\alpha]_D^{20}$ −10° (*c* 1.0, $CHCl_3$); IR ($CDCl_3$) 2104 (N_3), 1745 (C═O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.02 (dd, 1H, $J = 8.5$, 2 Hz, H_1), 4.89 (dd, 1H, $J = 9.5$, 3.5 Hz, H_4), 4.22−4.17 (m, 3H, H_3, H_6, $H_{6'}$), 4.12−4.05 (m, 1H, H_5), 2.13 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 2.03 (ddd, 1H, $J = 14$, 4, 2 Hz, H_{2e}), 1.64 (m, 1H, $J = 14$, 8.5, 3.5 Hz, H_{2a}), 0.88 (s, 9H, *t*-Bu), 0.10 (s, 6H, CH_3−Si); MS (CI) *m*/*z* 405 $(M + NH_4)^+$. Anal. ($C_{16}H_{19}N_3O_6S$) C, H, N.

Compound **4**, syrup: R_f 0.45 (4:1 cyclohexane/EtOAc); $[\alpha]_D^{20}$ +10° (*c* 1.4, $CHCl_3$); IR ($CDCl_3$) 2103 (N_3), 1746 (C═O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.86 (dd, 1H, $J = 9.5$, 1.5 Hz, H_1), 4.86 (t, 1H, $J = 9.5$, 9.5 Hz, H_4), 4.20 (dd, 1H, $J = 12$, 6 Hz, H_6), 4.10 (dd, 1H, $J = 12$, 2.5 Hz, $H_{6'}$), 3.62−3.55 (m, 2H, H_3, H_5), 2.25 (ddd, 1H, $J = 12.5$, 4.5, 1.5 Hz, H_{2e}), 2.15 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 1.72 (m, 1H, $J = 12.5$, 12.5, 9.5 Hz, H_{2a}), 0.92 (s, 9H, *t*-Bu), 0.14 (s, 3H, CH_3−Si), 0.13 (s, 3H, CH_3−Si); MS (CI): *m*/*z* 405 $(M + NH_4)^+$. Anal. ($C_{16}H_{19}N_3O_6S$) C, H, N.

***tert*-Butyldimethylsilyl 3-Azido-2,3-dideoxy-β-D-*ribo*-hexopyranoside (5).** To a stirred solution of **3** (1.1 g, 2.8 mmol) in anhydrous MeOH (15 mL) kept under argon was

added a 1 M solution of sodium methoxide (0.7 mL). After stirring for 1.5 h at room temperature and subsequent neutralization by addition of ion-exchange resin IRC 50 S, filtration, followed by concentration under reduced pressure, afforded **5** (0.87 g, 99%) as crystals: R_f 0.32 (2:1 cyclohexane/EtOAc); mp 70−72 °C; $[\alpha]_D^{20}$ +15° (*c* 1.0, $CHCl_3$); IR ($CDCl_3$) 3599, 3441 (OH), 2119 (N_3) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.04 (dd, 1H, J = 9, 2 Hz, H_1), 4.07 (q, 1H, J = 3.5, 3.5, 3.5 Hz, H_3), 3.82−3.78 (m, 3H, H_4, H_6, $H_{6'}$), 3.67 (m, J = 9.5, 4, 4 Hz, H_5), 2.98 (m, OH), 2.03 (ddd, 1H, J = 14, 3.5, 2 Hz, H_{2e}), 1.75 (ddd, J = 14, 9, 3.5 Hz, H_{2a}), 0.89 (s, 9H, *t*-Bu), 0.10 (s, 6H, CH_3−Si); MS (CI) *m*/*z* 304 $(M + H)^+$, 321 $(M + NH_4)^+$. Anal. ($C_{12}H_{25}N_3O_4Si$) C, H, N.

***tert*-Butyldimethylsilyl 3-Azido-2,3-dideoxy-β-D-*arabino*-hexopyranoside (6).** Obtained in 97% yield from **4** (3 g, 7,7 mmol) by similar treatment as above for **5**: R_f 0.36 (2:1 cyclohexane/EtOAc); mp 70−72 °C (EtOAc); $[\alpha]_D^{20}$ −26° (*c* 1, $CHCl_3$); IR ($CDCl_3$) 3598, 3445 (OH), 2103 (N_3) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.85 (dd, 1H, J = 9, 2 Hz, H_1), 3.82−3.78 (m, 2H, H_6, $H_{6'}$), 3.52 (t, 1H, J = 9, 9 Hz, H_4), 3.55−3.42 (m, 1H, H_3), 3.30 (m, 1H, J = 9, 3.5, 3.5 Hz, H_5), 2,15 (ddd, 1H, J = 12.5, 4.5, 2 Hz, H_{2e}), 1.62 (m, 1H, J = 12.5, 12.5, 9 Hz, H_{2a}), 0.89 (s, 9H, *t*-Bu), 0.11 (s, 3H, CH_3−Si), 0.10 (s, 3H, CH_3−Si); MS (CI) *m*/*z* 304 $(M + H)^+$, 321 $(M + NH_4)^+$. Anal. ($C_{12}H_{25}N_3O_4Si$) C, H, N.

***tert*-Butyldimethylsilyl 3-Azido-4,6-di-*O*-chloroacetyl-2,3-dideoxy-β-D-*ribo*-hexopyranoside (7).** Chloroacetyl chloride (920 μL, 7.9 mmol) was added under argon atmosphere to a cooled solution (−10 °C) of **5** (0.87 g, 2.9 mmol) in a mixture of anhydrous CH_2Cl_2 (10 mL) and pyridine (930 μL). After stirring for 4 h at −10 °C, the crude mixture was poured into H_2O (20 mL). The separated aqueous layer was extracted twice with CH_2Cl_2 (2 × 10 mL), and the combined organic phases were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. This afforded a residue (1.3 g) which was purified by flash chromatography (9:1 cyclohexane/EtOAc), giving pure **7** (1.2 g, 92%) as a syrup: R_f 0.53 (4:1 cyclohexane/EtOAc); $[\alpha]_D^{20}$ +14° (*c* 1.6, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 5.09 (dd, 1H, J = 9, 2 Hz, H_1), 4.98 (dd, 1H, J = 9.5, 3.5 Hz, H_4), 4.31 (d, 2H, H_6, $H_{6'}$), 4.20−4.10 (m, 1H, J = 9.5, 4, 4 Hz, H_5), 4.10 (d, 4H, $ClCH_2CO$), 4.07 (q, 1H, J = 3.5, 3.5, 3.5 Hz, H_3), 2.07 (ddd, 1H, J = 14, 3.5, 2 Hz, H_{2e}), 1.85 (ddd, 1H, J = 14, 9, 3.5 Hz, H_{2a}), 0.89 (s, 9H, *t*-BuSi), 0.11 (s, 6H, CH_3−Si); MS (CI) *m*/*z* 474 $(M + NH_4)^+$.

***tert*-Butyldimethylsilyl 3-Azido-4,6-di-*O*-chloroacetyl-2,3-dideoxy-β-D-*arabino*-hexopyranoside (8).** It was obtained from **6** (0.6 g, 1.9 mmol) in 92% yield, as **7** from **5**, syrup: R_f 0.50 (4:1 cyclohexane/EtOAc); $[\alpha]_D^{20}$ −12° (*c* 1.5, $CHCl_3$); IR ($CDCl_3$) 2104 (N_3), 1763 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.90 (t, 1H, J = 10, 10 Hz, H_4), 4.87 (dd, 1H, J = 9.5, 2 Hz, H_1), 4.28 (m, 2H, H_6, $H_{6'}$), 4.11 (d, 4H, $ClCH_2CO$), 3.70−3.65 (m, 1H, H_5), 3.65−3.57 (m, 1H, J = 13, 10, 5 Hz, H_3), 2.07 (ddd, 1H, J = 13, 5, 2 Hz, H_{2e}), 1.85 (ddd, 1H, J = 13, 13, 9.5 Hz, H_{2a}), 0.90 (s, 9H, *t*-Bu), 0.12 (s, 3H, CH_3−Si), 0.11 (s, 3H, CH_3−Si); MS (CI) *m*/*z* 474 $(M + NH_4)^+$.

***tert*-Butyldimethylsilyl 3-Azido-2,3-dideoxy-4,6-*O*-ethylidene-β-D-*ribo*-hexopyranoside (9).** To a solution of **5** (5.8 g, 19.1 mmol) in acetonitrile (100 mL) were added 1,1-diethoxyethane (27.2 mL, 191 mmol) and *p*-TsOH (1.1 g, 5.7 mmol). The mixture was stirred at room temperature for 1 h and then diluted with EtOAc (200 mL). The organic layer was separated and washed successively with an aqueous saturated solution of $NaHCO_3$ (200 mL) and with H_2O (200 mL). After the mixture was dried over anhydrous $MgSO_4$, the concentration was reduced under pressure followed by flash chromatography (95:5 cyclohexane/EtOAc), giving 3 g (48%) of **9** as a syrup: R_f 0.77 (4:1 cyclohexane/EtOAc); $[\alpha]_D^{20}$ −35° (*c* 1.1, $CHCl_3$); IR ($CDCl_3$) 2115 (N_3) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.02 (dd, 1H, J = 9, 2 Hz, H_1), 4.71 (q, 1H, J = 5 Hz, CH−CH_3), 4.13−4.07 (m, 2H, H_3, H_6), 3.81 (m, 1H, H_5), 3.56−3.48 (m, 2H, H_4, $H_{6'}$), 1.98 (m, 1H, H_{2e}), 1.75 (ddd, 1H, H_{2a}), 1.35 (d, 3H, J = 5 Hz, CH_3−CH), 0.87 (s, 9H, *t*-Bu), 0.09 (s, 6H, CH_3−Si); MS (CI) *m*/*z* 330 $(M + H)^+$, 347 $(M + NH_4)^+$. Anal. ($C_{14}H_{27}N_3O_4Si$) C, H, N.

***tert*-Butyldimethylsilyl 3-Azido-2,3-dideoxy-4,6-*O*-ethylidene-β-D-*arabino*-hexopyranoside (10).** A solution of **6** (0.2 g, 0.6 mmol) in acetonitrile (5 mL) in the presence of 1,1-diethoxyethane (0.94 mL, 6.6 mmol) and *p*-TsOH (0.015 g, 0.08 mmol) was treated as described above for the preparation of **9**. This led to **10** (0.19 g, 85%) after flash chromatography (95:5 cyclohexane/EtOAc), syrup: R_f 0.89 (7:3 cyclohexane/EtOAc); $[\alpha]_D^{20}$ −19° (*c* 1.1, $CHCl_3$); IR ($CDCl_3$) 2114 (N_3) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.80 (dd, 1H, J = 9.5, 2 Hz, H_1), 4.68 (q, 1H, J = 5 Hz, CH−CH_3), 4.03 (dd, 1H, J = 10, 4.5 Hz, H_6), 3.60−3.54 (m, 1H, H_3), 3.50 (t, 1H, J = 10, 10 Hz, $H_{6'}$), 3.29−3.16 (m, 2H, H_4, H_5), 2.10 (ddd, 1H, J = 13, 4.5, 2 Hz, H_{2e}), 1.55 (ddd, 1H, J = 13, 13, 9.5 Hz, H_{2a}), 1.30 (d, 3H, J = 5 Hz, CH_3−CH), 0.82 (s, 9H, *t*-Bu), 0.04 (s, 6H, CH_3−Si). Anal. ($C_{14}H_{27}N_3O_4Si$), C, H, N.

4-Demethyl-epipodophyllotoxin (12). To a solution of podophyllotoxin **11** (3.22 g, 7.77 mmol) in anhydrous CH_2Cl_2 (100 mL) kept under argon, a solution of trimethylsilyl iodide (3.30 mL, 23.31 mmol) in CH_2Cl_2 (10 mL) was added dropwise at 0 °C over a period of 0.5 h. The mixture was stirred for an additional 4.5 h at 0 °C, and the reaction was quenched with acetone/H_2O (1/1, 100 mL) and then $BaCO_3$ (1.5 g). Half an hour later, the mixture was extracted with CH_2Cl_2 (100 mL). The organic layer was separated and washed with 10% aqueous solution of sodium thiosulfate (500 mL). After the mixture was dried over anhydrous $MgSO_4$ and concentrated in vacuo, flash chromatography (92:8 CH_2Cl_2/acetone) afforded 4′-demethyl-epipodophyllotoxin **12** (2.23 g, 72%) pure enough for the next step. A sample was recrystallized from acetone−isopropyl ether: mp 248−250 °C; $[\alpha]_D^{20}$ −69° (*c* 0.4, $CHCl_3$); lit.[23] mp 244−249 °C (acetone); $[\alpha]_D^{20}$ −69° (*c* 0.63, $CHCl_3$).

4′-Benzyloxycarbonyl-epipodophyllotoxin (13). A solution of the crude material **12** (2 g, 4.8 mmol) was dissolved into anhydrous CH_2Cl_2 (120 mL) and cooled to 0 °C. To this solution were added Et_3N (1.2 mL, 8.8 mmol) and benzylchloroformate (1.1 mL, 7.2 mmol). After the crude mixture was stirred for 2 h at 0 °C, it was washed twice with water and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography (96:4 then 92:8 CH_2Cl_2/acetone) led to **13** (2.6 g, 97%) which recrystallized from acetone−isopropyl ether: mp 205−206 °C; $[\alpha]_D^{20}$ −43.5° (*c* = 1, $CHCl_3$); lit.[23b] mp 205−207 °C; $[\alpha]_D^{20}$ −44.5° (*c* = 0.506, $CHCl_3$); R_f 0.37 (92:8 CH_2Cl_2/acetone).

4-*O*-(3″-Azido-2″,3″-dideoxy-4″,6″-di-*O*-chloroacetyl-β-D-*ribo*-hexopyranosyl)-4′-benzyloxycarbonyl-epipodophyllotoxin (14). To a cooled (−35 °C) mixture of **7** (0.235 g, 0.51 mmol) and **13** (0.25 g, 0.51 mmol) in anhydrous CH_2Cl_2 (10 mL) in the presence of 4 Å powdered molecular sieves (1.4 g) was added trimethylsilyl triflate (270 μL, 1.4 mmol). After it was stirred for 22 h at −35 °C, the reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed successively with a citrate buffer (pH 5, 20 mL) and with H_2O (20 mL). Usual workup followed by flash chromatography (7:3 cyclohexane/EtOAc) led to **14** (0.2 g, 50%) as a crystalline residue: mp 95 °C; R_f 0.69 (1:1 cyclohexane/EtOAc); $[\alpha]_D^{20}$ −69° (*c* 1, $CHCl_3$); IR ($CDCl_3$) 2105 (N_3), 1768 (C=O) cm^{-1}; MS (CI) *m*/*z* 875 $(M + NH_4)^+$.

4-*O*-(3″-Azido-2″,3″-dideoxy-β-D-*ribo*-hexopyranosyl)-4′-benzyloxycarbonyl)-epipodophyllotoxin (15). To a solution of the azido glycoside **14** (0.2 g, 0.02 mmol) in a mixture of CH_2Cl_2/MeOH (2/1, 6 mL) kept under argon atmosphere was added Amberlite IRA 410 ion-exchange (OH^-) resin. After it was stirred for 1.5 h at room temperature, the reaction mixture was filtered, and the filtrate was washed with a solution of phosphate buffer (pH 7, 5 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure, giving **15** (0.16 g, 97%) as a crystalline residue: mp 140 °C; R_f 0.20 (97:3 CH_2Cl_2/MeOH); $[\alpha]_D^{20}$ −67° (*c* = 1.2, $CHCl_3$); IR ($CDCl_3$) 3614 (OH), 2115 (N_3), 1768 (C=O) cm^{-1}; MS (CI) *m*/*z* 723 $(M + NH_4)^+$. Anal. ($C_{35}H_{35}N_3O_{13}$) C, H, N.

4-*O*-(3″-Azido-2″,3″-dideoxy-4″,6″-*O*-ethylidene-β-D-*ribo*-hexopyranosyl)-4′-benzyloxycarbonyl-epipodophyllo-

toxin (16). (1) From 15. Acetalation of **15** (0.16 g, 0.23 mmol) (cf. preparation of **9** and **10**) afforded **16** (0.11 g, 66%).

(2) From 9 and 13 in the Presence of Trimethylsilyltriflate. A mixture of **9** (0.27 g, 0.82 mmol) and **13** (0.44 g, 0.82 mmol) was treated under conditions similar to those described for the preparation of **14**, giving **16** in 36% yield (0.22 g) after flash chromatography using cyclohexane/EtOAc (65:35) as eluent.

(3) From 9 and 13 in the Presence of $BF_3 \cdot Et_2O$. To a cooled (−15 °C) solution of **13** (1.85 g, 3.46 mmol) and **9** (1.20 g, 3.64 mmol) in anhydrous CH_2Cl_2 (100 mL) was added $BF_3 \cdot Et_2O$ (425 μL, 3.46 mmol). After the solution was stirred for 2 h, it was diluted with CH_2Cl_2 (100 mL) and poured into a satured aqueous solution of $NaHCO_3$ (200 mL). The organic layer was separated and dried over anhydrous $MgSO_4$. Flash chromatography (65:35 cyclohexane/EtOAc) afforded **16** (1.19 g, 47%) which was recrystallized from Et_2O/hexane: mp 139 °C; R_f 0.41 (6:4 cyclohexane/EtOAc); $[\alpha]_D^{20}$ −101° (*c* 1.1, $CHCl_3$); IR ($CDCl_3$) 2114 (N_3), 1768 (C═O) cm^{-1}; MS (CI) *m*/*z* 749 (M + NH_4)$^+$. Anal. ($C_{37}H_{37}N_3O_{13}$) C, H, N.

4-*O*-(3″-Amino-2″,3″-dideoxy-4″,6″-*O*-ethylidene-*β*-D-*ribo*-hexopyranosyl)-epipodophyllotoxin (17). To a solution of **16** (0.26 g, 0.35 mmol) were added EtOAc (15 mL), triethylamine (20 μL), and palladium-on-charcoal 10% (0.15 g). After stirring for 2 h at room temperature under hydrogen atmosphere, the catalyst was eliminated by filtration. The filtrate, concentrated under reduced pressure, was purified by flash chromatography (98.5:1.5 then 96:4 CH_2Cl_2/MeOH), affording **17** (0.18 g, 88%) as a crystalline product: mp 188 °C; R_f 0.39 (95:5 CH_2Cl_2/MeOH); $[\alpha]_D^{20}$ −100° (*c* 1.05, $CHCl_3$); IR ($CDCl_3$) 3541 (NH_2, OH), 1774 (C═O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.86 (s, 1H, H_5), 6.51 (s, 1H, H_8), 6.25 (s, 2H, $H_{2'}$, $H_{6'}$), 5.97 (d, 1H, O−CH−O), 5.93 (d, 1H, O−CH−O), 5.38 (dd, 1H, *J* = 9, 2 Hz, $H_{1''}$), 4.91 (d, 1H, *J* = 3.4 Hz, H_4), 4.78 (q, 1H, *J* = 5 Hz, $H_{7''}$), 4.57 (d, 1H, *J* = 5.2 Hz, H_1), 4.42 (dd, 1H, *J* = 10.5, 9 Hz, H_{9a}), 4.19 (t, 1H, *J* = 9, 8 Hz, H_{9b}), 4.16 (dd, 1H, *J* = 10, 5 Hz, $H_{6''}$), 4.02−3.94 (m, 1H, $H_{5''}$), 3.74 (s, 3H, CH_3O), 3.59 (m, 1H, $H_{3''}$), 3.53 (t, 1H, *J* = 10, 10 Hz, $H_{6'''}$), 3.42 (dd, 1H, *J* = 9.5, 3.5 Hz, $H_{4''}$), 3.22 (dd, 1H, *J* = 14, 5.2 Hz, H_2), 2.83 (m, 1H, H_3), 1.90 (m, 1H, $H_{2''e}$), 1.73 (m, 1H, *J* = 13.5, 9, 3.5 Hz, $H_{2''a}$), 1.35 (d, 3H, *J* = 5 Hz, CH_3); MS (CI) *m*/*z* 572 (M + H)$^+$, 589 (M + NH_4)$^+$. Anal. (+ H_2O) ($C_{29}H_{33}NO_{11}$) C, H, N.

4-*O*-(3″-Azido-2″,3″-dideoxy-4″,6″-di-*O*-chloroacetyl-α-D-*arabino*-hexopyranosyl)-4′-benzyloxycarbonyl-epipodophyllotoxin (18). Trimethylsilyl triflate (32.6 mL, 0.18 mmol) was added to a cooled (−35 °C) solution of **13** (0.1 g, 0.18 mmol) and **8** (0.85 g, 0.18 mmol) in anhydrous CH_2Cl_2 (10 mL) containing 4 Å powdered molecular sieves (0.6 g). After the mixture was stirred for 48 h at −35 °C, the reaction was quenched by addition of citrate buffer (pH 5, 10 mL). The separated organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. Flash chromatography of the crude residue (2:1 cyclohexane/EtOAc) afforded **18** as a crystalline compound (0.1 g, 65%): R_f 0.58 (1:1 cyclohexane/EtOAc); IR ($CDCl_3$) 2107 (N_3), 1767 (C═O) cm^{-1}; MS (CI) *m*/*z* 875 (M + NH_4)$^+$.

4-*O*-(3″-Azido-2″,3″-dideoxy-α-D-*arabino*-hexopyranosyl)4′-benzyloxycarbonyl-epipodophyllotoxin (19). Azido glycoside **18** (0.074 g, 0.08 mmol) was treated under conditions similar to those described for the preparation of **15** from **14**, giving **19** (0.06 g, 98%) as a crystalline residue: mp 120 °C (from pentane); R_f 0.28 (93:7 CH_2Cl_2/MeOH); $[\alpha]_D^{20}$ +26° (*c* 1, $CHCl_3$); IR ($CDCl_3$) 3603 (OH), 2106 (N_3), 1769 (C═O) cm^{-1}; MS (CI) *m*/*z* 706 (M + H)$^+$, 723 (M + NH_4)$^+$. Anal. ($C_{35}H_{35}N_3O_{13}$) C, H, N.

4-*O*-(3″-Azido-2″,3″-dideoxy-4″,6″-*O*-ethylidene-*β*-D-*ribo*-hexopyranosyl)-4′-benzyloxycarbonyl-epipodophyllotoxin (20). Acetalation of **19** (0.12 g, 0.17 mmol) (cf. preparation of **9** and **10**) afforded **20** (0.112 g, 90%) as a crystalline product: mp 137 °C; R_f 0.57 (1:1 cyclohexane/EtOAc); $[\alpha]_D^{20}$ +30° (*c* 1, $CHCl_3$); IR ($CDCl_3$) 2108 (N_3), 1768 (C═O) cm^{-1}; MS (CI) *m*/*z* 749 (M + NH_4)$^+$. Anal. ($C_{37}H_{37}N_3O_{13}$) C, H, N.

4-*O*-(3″-Amino-2″,3″-dideoxy-4″,6″-*O*-ethylidene-α-D-*arabino*-hexopyranosyl)-epipodophyllotoxin (21). Azido glycoside **20** (0.13 g, 0.18 mmol) was treated under conditions similar to those described for the preparation of **19**, giving **21** (0.08 g, 75%) as a crystalline residue: mp 255 °C; R_f 0.28 (95:5 CH_2Cl_2/MeOH); $[\alpha]_D^{20}$ +7° (*c* 1, $CHCl_3$); IR ($CDCl_3$) 3540 (NH_2, OH), 1779 (C═O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.86 (s, 1H, H_5), 6.49 (s, 1H, H_8), 6.25 (s, 2H, $H_{2'}$, $H_{6'}$), 5.97 (d, 2H, O−CH_2−O), 4.96 (d, 1H, *J* = 3.5 Hz, $H_{1''}$), 4.74−4.69 (m, 2H, H_4, $H_{7''}$), 4.60 (d, 1H, *J* = 5.3 Hz, H_1), 4.36 (t, 1H, *J* = 8, 8 Hz, H_{9a}), 4.09−4.04 (m, 2H, H_{9b}, $H_{6''}$), 3.52 (s, 3H, CH_3O), 3.53−3.49 (m, 2H, $H_{5''}$, $H_{6'''}$), 3.32 (dd, 1H, *J* = 14, 5.3 Hz, H_2), 3.21−3.17 (m, 1H, $H_{3''}$), 3.10 (t, 1H, *J* = 9, 9 $H_{4''}$), 2.86 (m, 1H, H_3), 2.18 (dd, 1H, *J* = 13.5, 4.5 Hz, $H_{2''e}$), 1.75 (m, 1H, *J* = 13.5, 12, 3.5 Hz, $H_{2''a}$), 1.31 (d, 3H, *J* = 5 Hz, CH_3); MS (CI) *m*/*z* 572 (M + H)$^+$, 589 (M + NH_4)$^+$. Anal. ($C_{29}H_{33}NO_{11}$) C, H, N.

2,3-Dideoxy-4,6-*O*-ethylidene-D-*threo*-hex-2-enopyranose (22). To a solution of **10** (0.50 g, 1.5 mmol) in THF (10 mL) was added Bu_4NF (1.8 mL, 1.8 mmol) (1 M solution in THF). After it was stirred for 10 min at room temperature, the mixture was poured into H_2O (20 mL) and extracted with ether (6 × 20 mL). The organic layer was washed over anhydrous $MgSO_4$, concentrated under reduced pressure, and flash-chromatographed (7:3 cyclohexane/EtOAc) to give **22** as a syrup (0.15 g, 57%): R_f 0.39 (1:1 cyclohexane/EtOAc); IR ($CDCl_3$) 1692 (C═O), 1644 (C═C) cm^{-1}; ^{1}H NMR (90 MHz, $CDCl_3$) δ 9.60 (d, 1H, H aldehyde), 7.10 (dd, 1H, H_3), 6.40 (ddd, 1H, H_2), 4.20−4.00 (m, 2H, H_4, H_6), 3.80−3.50 (m, 2H, H_5, $H_{6'}$), 1.40 (d, 3H, CH_3).

***tert*-Butyldimethylsilyl 3-Amino-2,3-dideoxy-4,6-*O*-ethylidene-*β*-D-*arabino*-hexopyranoside (23).** A solution of **10** (2 g, 6 mmol) in EtOAc (30 mL) was stirred overnight under H_2 atmosphere (1 atm) in the presence of Et_3N (50 μL) and 10% of palladium on activated carbon (0.5 g). After removal of the catalyst by filtration, the filtrate was concentrated in vacuo, furnishing the corresponding amino sugar **23** (1.82 g, 98%) as a colorless syrup: R_f 0.23 (1:1 cyclohexane/EtOAc); $[\alpha]_D^{20}$ −28° (*c* 1.3, $CHCl_3$); IR ($CDCl_3$) 3428, 3370 (NH_2) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.89 (dd, 1H, *J* = 9, 2 Hz, H_1), 4.70 (q, 1H, *J* = 5 Hz, CH−CH_3), 4.07 (dd, 1H, *J* = 10, 5 Hz, H_6), 3.55 (t, 1H, *J* = 10, 10 Hz, $H_{6'}$), 3.22 (m, 1H, *J* = 10, 10, 5 Hz, H_5), 3.05−2.91 (m, 2H, H_3, H_4), 1.98 (m, 1H, *J* = 13, 4.5, 2 Hz, H_{2e}), 1.49 (m, 1H, H_{2a}), 1.33 (d, 3H, *J* = 5 Hz, CH_3−CH), 0.88 (s, 9H, *t*-Bu), 0.10 (s, 6H, CH_3−Si); MS (CI) *m*/*z* 304 (M + H)$^+$. Anal. ($C_{14}H_{29}NO_4Si$) C, H, N.

***tert*-Butyldimethylsilyl 3-Aminobenzyloxycarbonyl-2,3-dideoxy-4,6-*O*-ethylidene-*β*-D-*arabino*-hexopyranoside (24).** Benzyloxycarbonyl chloride (1.10 mL, 7.88 mmol) was added to a cooled (0−5 °C) solution of **23** (1.82 g, 6 mmol) in dichloromethane (30 mL) and in the presence of Et_3N (1.25 mL, 9 mmol). After the crude mixture was stirred for 3 h, it was poured into water (100 mL) and dichloromethane was subsequently added (100 mL). The organic layer was separated, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash chromatography (6:1 then 4:1 cyclohexane/EtOAc), giving **24** (1.9 g, 72%) as crystals: R_f 0.84 (1:1 cyclohexane/EtOAc); mp 102 °C (CH_2Cl_2); $[\alpha]_D^{20}$ −27° (*c* 1.14, $CHCl_3$); IR ($CDCl_3$) 3439 (NH), 1718 (C═O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37−7.30 (m, 4H, H_{ar}), 5.10 (s, 2H, CH_2Ph), 4.89 (dd, 1H, *J* = 9, 2 Hz, H_1), 4.71 (q, 1H, *J* = 5 Hz, CH−CH_3), 4.08 (dd, 1H, *J* = 10, 5 Hz, H_6), 3.82 (m, 1H, H_3), 3.55 (t, 1H, *J* = 10, 10 Hz, $H_{6'}$), 3.32 (m, 1H, *J* = 10, 10, 5 Hz, H_5), 3.18 (t, *J* = 10, 10 Hz, H_4), 2.45 (m, 1H, H_{2e}), 1.52 (m, 2H, *J* = 12, 12, 9 Hz, H_{2a}), 1.35 (d, 3H, *J* = 5 Hz, CH_3), 0.88 (s, 9H, *t*.Bu), 0.10 (s, 6H, CH_3−Si). Anal. ($C_{22}H_{35}NO_6Si$) C, H, N.

3-Aminobenzyloxycarbonyl-2,3-dideoxy-4,6-*O*-ethylidene-D-*arabino*-hexopyranose (25). To a solution of **24** (0.59 g, 1.35 mmol) in THF (30 mL) was added Bu_4NF (1.35 mL, 1.35 mmol; 1 M solution in THF). After it was stirred for 30 min at room temperature, the reaction mixture was poured into H_2O (60 mL) and extracted with CH_2Cl_2 (2 × 60 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to quantitatively give **25** (0.43

g) as a mixture of α- and β-isomers (in 1 to 1 ratio): R_f 0.24 (1:1 cyclohexane/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.05 (d, J = 3 Hz, H_{1eq}), 4.71 (dd, J = 9, 1.5 Hz, H_{1ax}); MS (CI) m/z 306 $(M + H-H_2O)^+$.

4-*O*-(3″-Aminobenzyloxycarbonyl-2″,3″-dideoxy-4″,6″-*O*-ethylidene-β-D-*arabino*-hexopyranosyl)-4′-benzyloxycarbonyl-epipodophyllotoxin (26). To the solution of amino sugar **24** (2 g, 4.57 mmol) in 100 mL of anhydrous CH_2Cl_2 at −20 °C was added Bu_4NF (5.05 mL, 1.1 M solution in THF, 5.56 mmol). After reacting for 5 min at −20 °C, the temperature was slowly heightened until disappearance of the starting compound. The mixture was then cooled to −20 °C before successive addition of epipodophyllotoxin derivative **13** (2.57 g, 4.80 mmol) and BF_3·Et_2O (8.45 mL, 68.6 mmol). After it was stirred for 1 h at the same temperature, the reaction mixture was diluted with CH_2Cl_2 (100 mL) and poured into a saturated solution of $NaHCO_3$ (600 mL). The organic layer was dried over anhydrous $MgSO_4$, concentrated under reduced pressure, and the crude residue (6.2 g) was purified by flash chromatography (98:2 then 97:3 CH_2Cl_2/acetone), affording **26** (2.08 g, 54%) as crystals: mp 175 °C; R_f 0,60 (92:8 CH_2Cl_2/acetone); $[\alpha]_D^{20}$ −74° (*c* 1.09, $CHCl_3$); IR ($CDCl_3$) 3441 (NH), 1768 and 1723 (C═O) cm^{-1}; MS (CI) m/z 857 $(M + NH_4)^+$. Anal. ($C_{45}H_{45}NO_{15}$) C, H, N.

4-*O*-(3″-Amino-2″,3″-dideoxy-4″,6″-*O*-ethylidene-β-D-*arabino*-hexopyranosyl)-epipodophyllotoxin (27). To a solution of **26** (0.28 g, 0.33 mmol) in EtOAc (20 mL) were successively added triethylamine (30 μL) and 10% palladium on activated carbon (0.15 g). The reaction was left under hydrogen atmosphere and under atmospheric pressure and was stirred for 1.5 h at room temperature. The catalyst was then eliminated by filtration, and the filtrate, concentrated under reduced pressure, was purified by flash chromatography (97:3 CH_2Cl_2/MeOH), giving **27** (0.17 g, 90%) as a crystalline compound: mp 219 °C; R_f 0.31 (95:5 CH_2Cl_2/MeOH); $[\alpha]_D^{20}$ −120° (*c* 1.05, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.75 (s, 1H, H_5), 6.55 (s, 1H, H_8), 6.24 (s, 2H, $H_{2'}$, $H_{6'}$), 6.00 (d, 1H, O−CH−O), 5.98 (d, 1H, O−CH−O), 4.94 (d, 1H, J = 3.3 Hz, H_4), 4.85 (dd, 1H, J = 9, 2 Hz, $H_{1''}$), 4.75 (q, 1H, J = 5 Hz, $H_{7''}$), 4.59 (d, 1H, J = 5.2 Hz, H_1), 4.41 (dd, 1H, J = 10.5, 9 Hz, H_{9a}), 4.21 (t, 1H, J = 9, 8 Hz, H_{9b}), 4.15 (dd, 1H, J = 10, 5 Hz, $H_{6''}$), 3.75 (s, 3H, CH_3O), 3.57 (t, 1H, J = 10, 10 Hz, $H_{6'''}$), 3.30 (m, 1H, $H_{5''}$), 3.28 (dd, 1H, J = 14, 5.2 Hz, H_2), 3.05−2.99 (m, 2H, $H_{3''}$, $H_{4''}$), 2.88 (m, 1H, H_3), 2.05 (m, 1H, $H_{2''e}$), 1.51 (m, 1H, $H_{2''a}$), 1.36 (d, 3H, J = 5 Hz, CH_3); MS (CI) m/z 594 $(M + Na)^+$, 610 $(M + K)^+$. Anal. ($C_{29}H_{33}NO_{11}$) C, H, N.

4-*O*-(3″-Dimethylamino-2″,3″-dideoxy-4″,6″-*O*-ethylidene-β-D-*arabino*-hexopyranosyl)-epipodophyllotoxin (28). To a solution of **27** (0.19 g, 0.33 mmol) in CH_2Cl_2 (15 mL) were added successively formaldehyde (135 μL, 1.66 mmol) and sodium cyanoborohydride (0.085 g, 1.33 mmol). After the mixture was stirred for 0.75 h at room temperature, an additional amount of formaldehyde (135 μL) and sodium cyanoborohydride (0.085 g) was poured into the mixture with additional stirring for 0.75 h at room temperature. The reaction mixture was diluted in CH_2Cl_2 (30 mL) and washed with H_2O (40 mL).The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. Flash chromatography (97:3 CH_2Cl_2/MeOH) gave **28** (0.1 g, 51%) as crystals: mp 270 °C; R_f 0.40 (95:5 CH_2Cl_2/MeOH); $[\alpha]_D^{20}$ −121° (*c* 1.0, $CHCl_3$); IR ($CDCl_3$) 3541 (OH), 1774 (C═O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.76 (s, 1H, H_5), 6.55 (s, 1H, H_8), 6.25 (s, 2H, $H_{2'}$, $H_{6'}$), 6.00 (d, 1H, O−CH−O), 5.97 (d, 1H, O−CH−O), 4.95 (d, 1H, J = 3.2 Hz, H_4), 4.82 (dd, 1H, J = 9.5, 2 Hz, $H_{1''}$), 4.74 (q, 1H, J = 5 Hz, $H_{7''}$), 4.59 (d, 1H, J = 5.2 Hz, H_1), 4.42 (dd, 1H, J = 10.5, 9 Hz, H_{9a}), 4.21 (t, 1H, J = 9, 8 Hz, H_{9b}), 4.16 (dd, 1H, J = 10, 5 Hz, $H_{6''}$), 3.75 (s, 3H, CH_3O), 3.58 (t, 1H, J = 10, 10 Hz, $H_{6'''}$), 3.38 (t, 1H, J = 9, 9 Hz, $H_{4''}$), 3.35−3.25 (m, 2H, H_2, $H_{5''}$), 2.91−2.82 (m, 2H, H_3, $H_{3''}$), 2.33 (s, 6H, CH_3−N), 1.96 (m, 1H, $H_{2''e}$), 1.55 (m, 1H, J = 12.5, 12.5, 9.5 Hz, $H_{2''a}$), 1.38 (d, 3H, J = 5 Hz, CH_3); MS (CI) m/z 600 $(M + H)^+$.

4-*O*-(3″-*N*,*N*-Dimethylamino-2″,3″-dideoxy-4″,6″-*O*-ethylidene-β-D-*ribo*-hexopyranosyl)-epipodophyllotoxin (29). To a solution of **17** (0.029 g, 0.05 mmol) in acetonitrile (1 mL) were added successively formaldehyde (10.5 μL) and sodium cyanoborohydride (0.012 g). After it was stirred for 2 h at room temperature, the reaction mixture was diluted in CH_2Cl_2 (20 mL) and washed with H_2O (20 mL).The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue, submitted again to the above treatment, was purified by flash chromatography (97:3 CH_2Cl_2/MeOH) affording **29** (0.029 g, 95%) as crystals: mp 140 °C; R_f 0.5 (95:5 CH_2Cl_2/MeOH); $[\alpha]_D^{20}$ −85° (*c* 1.06, $CHCl_3$); IR ($CDCl_3$) 3539 (OH), 1773 (C═O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.80 (s, 1H, H_5), 6.52 (s, 1H, H_8), 6.25 (s, 2H, $H_{2'}$, $H_{6'}$), 5.98 (d, 1H, O−CH−O), 5.96 (d, 1H, O−CH−O), 5.21 (dd, 1H, J = 9.5, 1.5 Hz, $H_{1''}$), 4.88 (d, 1H, J = 3.4 Hz, H_4), 4.62−4.57 (m, 2H, H_1, $H_{7''}$), 4.43 (dd, 1H, J = 10.5, 9 Hz, H_{9a}), 4.22−4.02 (m, 3H, H_{9b}, $H_{5''}$, $H_{6''}$), 3.75 (s, 3H, CH_3O), 3.61 (t, 1H, J = 10, 10 Hz, $H_{6'''}$), 3.52 (dd, 1H, J = 9, 3 Hz, $H_{4''}$), 3.23 (dd, 1H, J = 14, 5.3 Hz, H_2), 2.85 (m, 1H, H_3), 2.62 (m, 1H, $H_{3''}$), 2.36 (s, 6H, CH_3N), 2.20 (m, 1H, $H_{2''e}$), 1.55 (m, 1H, $H_{2''a}$), 1.35 (d, 3H, J = 5 Hz, CH_3); MS (CI) m/z 600 $(M + H)^+$.

4-*O*-(3″-Cyanomethylamino-2″,3″-dideoxy-4″,6″-*O*-ethylidene-β-D-*ribo*-hexopyranosyl)-epipodophyllotoxin (30). To a solution of **17** (0.1 g, 0.18 mmol) in DMF (5 mL) were added successively Et_3N (180 μL, 1.29 mmol) and iodoacetonitrile (94 μL, 1.29 mmol). The reaction mixture was stirred for 27 h at room temperature, poured into H_2O (20 mL), and extracted with EtOAc (30 mL). The organic layer was washed with H_2O (4 × 20 mL), dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. Flash chromatography (92:8 CH_2Cl_2/acetone) gave **30** (0.1 g, 85%) and recovery material **17** (0.04 g, 15%). Compound **30**: R_f 0.25 (95:5 CH_2Cl_2/MeOH); $[\alpha]_D^{20}$ −86° (*c* 0.80, $CHCl_3$); IR ($CDCl_3$) 3543 (NH, OH), 1774 (C═O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.78 (s, 1H, H_5), 6.53 (s, 1H, H_8), 6.25 (s, 2H, $H_{2'}$, $H_{6'}$), 6.00 (d, 1H, O−CH−O), 5.99 (d, 1H, O−CH−O), 5.17 (dd, 1H, J = 9.5, 2 Hz, $H_{1''}$), 4.90 (d, 1H, J = 3.3 Hz, H_4), 4.75 (q, 1H, J = 5 Hz, $H_{7''}$), 4.59 (d, 1H, J = 5.3 Hz, H_1), 4.41 (dd, 1H, J = 10.5, 9 Hz, H_{9a}), 4.19 (t, 1H, J = 9, 8 Hz, H_{9b}), 4.14 (dd, 1H, J = 10, 5 Hz, $H_{6''}$), 3.88 (m, 1H, $H_{5''}$), 3.76 (s, 3H, CH_3O), 3.56−3.51 (m, 4H, $H_{4''}$, $H_{6'''}$, CH_2CN), 3.46 (m, 1H, $H_{3''}$), 3.25 (dd, 1H, J = 14, 5.3 Hz, H_2), 2.86 (m, 1H, H_3), 1.93 (m, 1H, $H_{2''e}$), 1.69 (m, 1H, J = 13, 9.5, 3 Hz, $H_{2''a}$), 1.35 (d, 3H, J = 5 Hz, CH_3); MS (CI) m/z 611 $(M + H)^+$, 628 $(M + NH_4)^+$.

4-*O*-(3″-Amino-*N*-ethylaminobenzyloxycarbonyl-2″,3″-dideoxy-4″,6″-*O*-ethylidene-β-D-*ribo*-hexopyranosyl)-epipodophyllotoxin (31). To a solution of **17** (0.173 g, 0.30 mmol) in DMF (10 mL) were added successively Et_3N (127 μL, 0.91 mmol) and *N*-benzyloxycarbonyl-2-iodoethylamine (0.28 g, 0.91 mmol). The reaction mixture was stirred for 5 days at room temperature, poured into H_2O (30 mL), and extracted with EtOAc (30 mL). The organic layer was washed with H_2O (5 × 20 mL), dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. Flash chromatography (97:3 CH_2Cl_2/MeOH) gave **31** (0.155 g, 68%) and recovery material **17** (0.055 g, 32%). Compound **31**: mp 110 °C; R_f 0.70 (95:5 CH_2Cl_2/MeOH); $[\alpha]_D^{20}$ −74° (*c* 1.17, $CHCl_3$); MS (CI) m/z 749 $(M + H)^+$.

4-*O*-(3″-Amino-*N*-(ethylamino)-2″,3″-dideoxy-4″,6″-*O*-ethylidene-β-D-*ribo*-hexopyranosyl)-epipodophyllotoxin (32). To a solution of **31** (0.15 g, 0.20 mmol) in a mixture of EtOAc and ethanol (1:1, 10 mL) were successively added triethylamine (30 μL) and 10% palladium on activated carbon (0.1 g). The reaction, kept under hydrogen atmosphere, was stirred for 1.5 h at room temperature in the presence of hydrogen at atmospheric pressure, and the catalyst was eliminated by filtration. The filtrate, concentrated under reduced pressure, was purified by flash chromatography (97:3 CH_2Cl_2/MeOH(NH_3)) to give **32** (0.1 g, 84%): mp 130 °C; R_f 0.22 (95:5 CH_2Cl_2/MeOH(NH_3)); $[\alpha]_D^{20}$ −77° (*c* 1, $CHCl_3$); IR ($CDCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.80 (s, 1H, H_5), 6.52 (s, 1H, H_8), 6.25 (s, 2H, $H_{2'}$, $H_{6'}$), 5.99 (d, 1H, O−CH−O), 5.96 (d, 1H, O−CH−O), 5.27 (dd, 1H, J = 9.5, 2 Hz, $H_{1''}$), 4.89 (d, 1H, J = 3.4 Hz, H_4), 4.77 (q, 1H, J = 5 Hz, $H_{7''}$), 4.58 (d, 1H, J = 5.2 Hz, H_1), 4.43 (dd, 1H, J = 10.5, 9 Hz, H_{9a}), 4.16 (t, 1H,

J = 9, 8 Hz, H_{9b}), 4.12 (dd, 1H, J = 10, 5 Hz, $H_{6'''}$), 3.98 (m, 1H, $H_{5''}$), 3.75 (s, 3H, CH_3O), 3.55−3.45 (m, 2H, $H_{4''}$, $H_{6'''}$), 3.25 (dd, 1H, J = 14, 5.2 Hz, H_2), 3.20 (m, 1H, $H_{3''}$), 2.90−2.74 (m, 3H, H_3, CH_2NH_2), 2.67 (t, 2H, CH_2NH), 2.02 (m, 1H, $H_{2''e}$), 1.55 (m, 1H, $H_{2''a}$), 1.35 (d, 3H, J = 5 Hz, CH_3); MS (CI) m/z 615 (M + H)$^+$.

4-*O*-(3″-Morpholino-2″,3″-dideoxy-4″,6″-*O*-ethylidene-*β*-D-*ribo*-hexopyranosyl)-epipodophyllotoxin (33). To a solution of **17** (0.06 g, 0.10 mmol) in DMF (2 mL) were added successively triethylamine (58 μL, 0.42 mmol) and diiodoethyl ether (0.5 g, 1.57 mmol). The reaction mixture was stirred for 68 h at room temperature in the dark and diluted with H_2O (30 mL) and EtOAc (30 mL). The organic layer was washed with H_2O (4 × 30 mL), dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. Flash chromatography (92:8 CH_2Cl_2/acetone) gave **33** (0.046 g, 68%) as a syrup: R_f 0.31 (92:8 CH_2Cl_2/acetone); $[\alpha]_D^{20}$ −98° (*c* 1.04, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.73 (s, 1H, H_5), 6.54 (s, 1H, H_8), 6.25 (s, 2H, $H_{2'}$, $H_{6'}$), 6.00 (d, 1H, O−CH−O), 5.97 (d, 1H, O−CH−O), 5.20 (dd, 1H, J = 9.5, 2 Hz, $H_{1''}$), 4.89 (d, 1H, J = 3.4 Hz, H_4), 4.62−4.57 (m, 2H, H_1, $H_{7''}$), 4.43 (dd, 1H, J = 10.5, 9 Hz, H_{9a}), 4.20 (t, 1H, J = 9, 8 Hz, H_{9b}), 4.16−4.08 (m, 2H, $H_{5''}$, $H_{6''}$), 3.76−3.66 (m, 7H, CH_2O, CH_3O), 3.57 (dd, 1H, J = 9, 3 Hz, $H_{4''}$), 3.48 (t, 1H, J = 12, 12 Hz, $H_{6'''}$), 3.23 (dd, 1H, J = 14, 5.2 Hz, H_2), 2.90−2.84 (m, 3H, H_3, CH−N), 2.80 (q, 1H, J = 3, 3, 3 Hz, $H_{3''}$), 2.42 (m, 2H, CH−N), 2.15 (m, 1H, $H_{2''e}$), 1.55 (ddd, 1H, J = 13, 9.5, 1 Hz, $H_{2''a}$), 1.33 (d, 3H, J = 5 Hz, CH_3); MS (CI) m/z 642 (M + H)$^+$.

***tert*-Butyldimethylsilyl 3-Azido-2,3-dideoxy-6-*O*-tosyl-*β*-D-*ribo*-hexopyranoside (34).** A solution of tosyl chloride (1.55 g, 8.15 mmol) in pyridine (10 mL) was added dropwise to a cooled solution (0 °C) of diol **5** (2.06 g, 6.79 mmol). After the solution was stirred for 1 h at 0 °C, it was allowed to reach room temperature and stirred for an additional 18 h. The reaction mixture was then extracted with CH_2Cl_2 (2 × 100 mL), and the organic layer was washed with H_2O (2 × 50 mL), dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash chromatography (8:2 cyclohexane/EtOAc), giving 2.02 g (65%) of **34** and starting material **5** (0.4 g, 26%). Compound **34**, syrup: R_f 0.46 (2:1 cyclohexane/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82−7.79, 7.37−7.34, (m, 4H, H_{ar}), 4.99 (dd, 1H, J = 9, 2 Hz, H_1), 4.21 (m, 2H, H_6, $H_{6'}$), 4.10 (m, 1H, H_3), 3.80−3.73 (m, 2H, H_4, H_5), 2.46 (s, 3H, CH_3), 2.07 (m, 1H, J = 14, 3.5, 2 Hz, H_{2e}), 1.78 (m, 1H, J = 14, 9, 3.5 Hz, H_{2a}), 0.90 (s, 9H, *t*-Bu), 0.09 (s, 6H, CH_3−Si); MS (CI) m/z 475 (M + NH_4)$^+$. Anal. ($C_{19}H_{31}N_3O_6SSi$) C, H, N.

***tert*-Butyldimethylsilyl 3-Azido-2,3,6-trideoxy-6-iodo-*β*-D-*ribo*-hexopyranoside (35).** A solution of **34** (2.02 g, 4.42 mmol) in 120 mL acetone was heated under reflux for 72 h in the presence of sodium iodide (2.65 g, 17.6 mmol). After cooling, the crude mixture was concentrated under reduced pressure to ca. 30 mL and then diluted with CH_2Cl_2 (100 mL). The solution was then washed with 10% aqueous solution of sodium thiosulfate and dried over anhydrous $MgSO_4$. Evaporation under reduced pressure followed by flash chromatography (8:2 cyclohexane/EtOAc) gave **35** (1.5 g, 82%) as a syrupy residue: R_f 0.50 (4:1 cyclohexane/EtOAc); $[\alpha]_D^{20}$ −30° (*c* 1.0, $CHCl_3$); IR ($CDCl_3$) 2123 (N_3) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.07 (dd, 1H, J = 9, 2 Hz, H_1), 4.08 (m, 1H, H_3), 3.58−3.48 (m, 3H, H_4, H_5, H_6), 3.25 (dd, 1H, J = 10, 7 Hz, $H_{6'}$), 2.15 (m, 1H, J = 14, 3.5, 2 Hz, H_{2e}), 1.85 (m, 1H, J = 14, 9, 3.5 Hz, H_{2a}), 0.92 (s, 9H, *t*-Bu), 0.19 (s, 3H, CH_3−Si), 0.18 (s, 3H, CH_3−Si); MS (CI) m/z 431 (M + NH_4)$^+$. Anal. ($C_{12}H_{24}N_3O_3ISi$) C, H, N.

***tert*-Butyldimethylsilyl 3-Azido-4-*O*-chloroacetyl-2,3,6-trideoxy-6-iodo-*β*-D-*ribo*-hexopyranoside (36).** Chloroacetyl chloride (395 μL, 5 mmol) was added to a cooled (−10 °C) solution of **35** (1.03 g, 2.5 mmol) in CH_2Cl_2 (20 mL) and pyridine (405 μL, 5 mmol). After stirring for 1 h at the same temperature, CH_2Cl_2 was added (30 mL). The organic solution was washed with water (3 × 20 mL) and dried over $MgSO_4$. After evaporation and flash chromatography (10:1 cyclohexane/EtOAc), compound **36** was isolated (1.1 g, 90%) as a syrup: R_f 0.46 (6:1 cyclohexane/EtOAc); $[\alpha]_D^{20}$ −14° (*c* 1.03, $CHCl_3$); IR ($CDCl_3$) 2110 (N_3), 1766 (C=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.08 (dd, 1H, J = 9, 2 Hz, H_1), 4.83 (dd, 1H, J = 9.5, 3.5 Hz, H_4), 4.23 (m, 1H, J = 3.5, 3.5, 3.5 Hz, H_3), 4.13 (s, 2H, $ClCH_2CO$), 3.90 (m, 1H, J = 9.5, 8, 3 Hz, H_5), 3.35 (dd, 1H, J = 11, 3 Hz, H_6), 3.15 (dd, 1H, J = 11, 8 Hz, $H_{6'}$), 2.07 (m, 1H, J = 14, 3.5, 2 Hz, H_{2e}), 1.86 (m, 1H, J = 14, 9, 3.5 Hz, H_{2a}), 0.92 (s, 9H, *t*-Bu), 0.19 (s, 3H, CH_3−Si), 0.18 (s, 3H, CH_3−Si); MS (CI) m/z 507 (M + NH_4)$^+$. Anal. ($C_{14}H_{25}N_3O_4ClISi$) C, H, N.

4-*O*-(3″-Azido-6″-iodo-4″-*O*-chloroacetyl-2″,3″,6″-trideoxy-*β*-D-*ribo*-hexopyranosyl)-4′-benzyloxycarbonyl-epipodophyllotoxin (37). To a mixture of **13** (1 g, 1.85 mmol) and **36** (1 g, 2.04 mmol) in anhydrous CH_2Cl_2 (100 mL) cooled to −15 °C was added BF_3·Et_2O (455 μL, 3.7 mmol). After reacting for 5 h (−15 °C → 0 °C), the reaction mixture was diluted with CH_2Cl_2 (100 mL) and poured into a solution of saturated $NaHCO_3$ (200 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude residue was purified by flash chromatography (7:3 cyclohexane/EtOAc), giving **37** (0.8 g, 48%) as crystals: mp 143 °C; R_f 0.58 (1:1 cyclohexane/EtOAc); $[\alpha]_D^{20}$ −85° (*c* 1.25, $CHCl_3$); IR ($CDCl_3$) 2103 (N_3), 1773 (C=O) cm^{-1}; MS (CI) m/z 909 (M + NH_4)$^+$. Anal. ($C_{37}H_{35}N_3O_{13}ClI$) C, H, N.

4-*O*-(3″,6″-Diazido-2″,3″,6″-trideoxy-4″-*O*-azidoacetyl-*β*-D-*ribo*-hexopyranosyl)-4′-benzyloxycarbonyl-epipodophyllotoxin (38). To a solution of **37** (0.45 g, 0.51 mmol) in DMF (10 mL) was added NaN_3 (0.1 g, 1.5 mmol). The reaction mixture was stirred for 64 h at room temperature and diluted with H_2O (30 mL) and EtOAc (30 mL). The organic layer was washed with H_2O (4 × 20 mL), dried over $MgSO_4$, and concentrated under reduced pressure. Flash chromatography (7:3 cyclohexane/EtOAc) afforded **38** (0.36 g, 90%): mp 120 °C; R_f 0.44 (6:4 cyclohexane/EtOAc); $[\alpha]_D^{20}$ −64° (*c* 1, $CHCl_3$); IR ($CDCl_3$) 2109 (N_3), 1767 (C=O) cm^{-1}; MS (CI) m/z 831 (M + NH_4)$^+$. Anal. ($C_{37}H_{35}N_9O_{13}$) C, H, N.

4-*O*-(3″,6″-Diazido-2″,3″,6″-trideoxy-*β*-D-*ribo*-hexopyranosyl)-4′-benzyloxycarbonyl-epipodophyllotoxin (39). To a solution of azido glycoside **38** (0.07 g, 0.08 mmol) in a CH_2Cl_2/MeOH mixture (2:1, 3 mL) was added Amberlite resin IRA 410 (OH^-). After reaction for 3 h at 20 °C, the reaction mixture was filtered and concentrated under reduced pressure to give **39** (0.06 g, 94%) as crystals: mp 125 °C; R_f 0.25 (6:4 cyclohexane/EtOAc); $[\alpha]_D^{20}$ −53° (*c* 1.04, $CHCl_3$); IR ($CDCl_3$) 2103 (N_3), 1768 (C=O) cm^{-1}; MS (CI) m/z 748 (M + NH_4)$^+$.

4-*O*-(3″,6″-Diamino-2″,3″,6″-trideoxy-*β*-D-*ribo*-hexopyranosyl)-epipodophyllotoxin (40). To a solution of **39** (0.11 g, 0.15 mmol) in EtOAc/ethanol (1:1, 10 mL) were successively added triethylamine (20 μL) and 10% palladium on activated carbon (0.07 g). After the mixture was stirred for 30 h at room temperature in the presence of hydrogen under atmospheric pressure, the catalyst was eliminated by filtration and the filtrate was concentrated under reduced pressure to afford **40** (0.078 g, 95%): mp 110 °C; R_f 0.06 (90:10 CH_2Cl_2/MeOH(NH_3)); $[\alpha]_D^{20}$ −86° (*c* 1.04, MeOH); IR ($CDCl_3$) 3544, 3406 (NH_2, OH), 1772 (C=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.83 (s, 1H, H_5), 6.53 (s, 1H, H_8), 6.26 (s, 2H, $H_{2'}$, $H_{6'}$), 5.99 (s, 1H, O−CH−O), 5.96 (s, 1H, O−CH−O), 5.22 (dd, 1H, J = 2, 9 Hz, $H_{1''}$), 4.91 (d, 1H, J = 3.4 Hz, H_4), 4.58 (d, 1H, J = 5.3 Hz, H_1), 4.47 (t, 1H, J = 9, 10.5 Hz, H_{9a}), 4.20 (t, 1H, J = 8, 9 Hz, H_{9b}), 3.76 (s, 3H, CH_3O), 3.70 (m, 1H, $H_{5''}$), 3.61 (dd, 1H, J = 4, 9 Hz, $H_{4''}$), 3.46 (m, 1H, $H_{3''}$), 3.26 (dd, 1H, J = 5.3, 14 Hz, H_2), 3.18 (dd, 1H, J = 5, 12 Hz, $H_{6''}$), 2.96 (t, 1H, J = 8, 12 Hz, $H_{6'''}$), 2.86 (m, 1H, H_3), 1.82 (m, 1H, $H_{2''e}$), 1.73 (m, 1H, $H_{2''a}$); MS (CI) m/z 545 (M + H)$^+$. Anal. ($C_{27}H_{32}N_2O_{10}$) C, H, N.

4-*O*-(3″-Azido-6″-iodo-2″,3″,6″-trideoxy-*β*-D-*ribo*-hexopyranosyl)-4′-benzyloxycarbonyl-epipodophyllotoxin (41). To a solution of the azido glycoside **37** (0.26 g, 0.29 mmol) in a CH_2Cl_2/MeOH mixture (2:1, 15 mL) was added Amberlite resin IRA 410 (OH^-). After reacting for 3 h at 20 °C, the reaction mixture was filtered and concentrated under reduced pressure to give **41** (0.22 g, 94%) as crystals: mp 115 °C; R_f

0.46 (1:1 cyclohexane/EtOAc); $[\alpha]_D^{20}$ −57° (*c* 1.02, $CHCl_3$); IR ($CDCl_3$) 2105 (N_3), 1768 (C═O) cm^{-1}; MS (CI) *m/z* 833 $(M + NH_4)^+$.

4-*O*-(3″-Amino-2″,3″,6″-trideoxy-*β*-D-*ribo*-hexopyranosyl)-epipodophyllotoxin (42). To a solution of **41** (0.20 g, 0.25 mmol) in EtOAc (10 mL) were successively added triethylamine (100 μL) and 10% palladium on activated carbon (0.2 g). After stirring for 30 h at room temperature in the presence of hydrogen under atmospheric pressure, the catalyst was eliminated by filtration, and the filtrate was concentrated under reduced pressure. Flash chromatography (92:8 CH_2Cl_2/MeOH) afforded **42** (0.05 g, 38%) as crystals: mp 140 °C; R_f 0.33 (90:10 CH_2Cl_2/MeOH); $[\alpha]_D^{20}$ −90° (*c* 0.5, $CHCl_3$); IR ($CDCl_3$) 3543 (NH_2, OH), 1768 (C═O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.82 (s, 1H, H_5), 6.54 (s, 1H, H_8), 6.26 (s, 2H, $H_{2'}$, $H_{6'}$), 6.00 (d, 1H, O−CH−O), 5.97 (d, 1H, O−CH−O), 5.01 (dd, 1H, J = 2, 9 Hz, $H_{1''}$), 4.96 (d, 1H, J = 3.3 Hz, H_4), 4.59 (d, 1H, J = 5.3 Hz, H_1), 4.48 (dd, 1H, J = 9, 10.5 Hz, H_{9a}), 4.24 (t, 1H, J = 8, 9 Hz, H_{9b}), 3.76 (s, 3H, CH_3O), 3.65 (m, 1H, $H_{5''}$), 3.35 (m, 1H, J = 4, 4, 4 Hz, $H_{3''}$), 3.31−3.25 (m, 2H, H_2, $H_{4''}$), 2.85 (m, 1H, H_3), 1.90 (m, 1H, J = 2, 4, 14 Hz, $H_{2''e}$), 1.78 (m, 1H, J = 4, 9, 14 Hz, $H_{2''a}$), 1.34 (d, 3H, J = 6 Hz, CH_3); MS (CI) *m/z* 530 $(M + H)^+$.

In Vivo Studies. Female DBA/2 (Iffa Credo) and hybrid CDF_1 (Balb/c × DBA/2) mice were used for implanting with the murine P388 leukemia. The P388 leukemia was passaged as ip implants. For chemotherapy testing, tumors were transplanted in the same strain or in the appropriate F_1 hybrid. All mice were over 18 g at the start of each study. One million P388 cells/mouse were implanted iv in CDF_1 mice on day 0. After randomization into treatment cages, test compounds were administered ip on day 1 as single doses, taken from the geometric series 2.5, 10, 40, 160 mg/kg. Mice were checked daily for any adverse clinical reactions, which were then noted and the day of death was recorded. Mice were weighed on days 1, 4, and 8. Evaluation of antitumor activity was measured in terms of life span. An increase of life span was defined as the median survival of treated mice/median survival of control mice × 100 (T/C, %). According to NCI standard criteria for the P388 tumor model, 125% ≤ T/C ≤ 175% is the minimum level for activity.

In Vitro Studies. Growth Inhibition Assays Using L1210 Cells. The murine lymphocytic L1210 leukemia cell line was obtained from the American Type Culture Collection (ATCC). Cells were cultured in RPMI 1640 medium, supplemented with 10% heat-inactivated horse serum, 4 mM L-glutamine, 100 U/L penicillin, 100 μg/mL streptomycin, and 1.25 μg/mL fungizone at 37 °C in an humidified atmosphere of 5% CO_2 in air. Cells were split twice a week and used for studies over 40 in vitro passages. Under these conditions the population doubling time of these cells was 8−10 h. Effects of test compounds on L1210 cell proliferation were determined using a standard growth inhibition assay. Exponentially growing L1210 cells (1.5×10^5 cells/well) in a 24-well plate were exposed to a range of concentrations of test compounds for 48 h, after which the control and treated cells were counted using an electronic particle counter. Computerized data was processed using an in-house custom-designed program. The IC_{50} values, i.e., the concentration of test compound to reduce the cell number to 50% of that obtained with control cells, were then determined from replicates of 6.

Effects of Compounds on Microtubule Polymerization or Depolymerization. Microtubular proteins were prepared from sheep brain by three cycles of polymerization and depolymerization. The final pellet was resuspended in extraction buffer (0.1 M PIPES, pH 6.6, 0.5 mM $MgCl_2$, 0.1 mM EDTA, 1 μM EGTA) and stored in liquid nitrogen before use or further purification. Protein concentrations were determined using bovine serum albumin as a standard. In vitro microtubule polymerization or depolymerization was followed turbidimetrically as described by Gaskin et al.[35] Turbidity experiments were conducted with 2.3 mg/mL solutions of microtubular proteins in polymerization buffer containing 0.1 M PIPES, pH 6.6, 0.5 mM $MgCl_2$, 0.1 mM EDTA, 1 μM EGTA, and 2 mM GTP. Reaction mixtures containing 2 mg/mL of purified tubulin in 0.7 M monosodium glutamate, pH 6.6, 1 mM $MgCl_2$, and 0.4% DMSO were preincubated at 37 °C for 15 min without GTP, chilled on ice for 10 min, and then 0.4 mM GTP was added at the same time as the test compound.[36] Adsorbance of the solution of tubulin was determined at 350 nm. The IC_{50} values for inhibition of assembly or disassembly were those concentrations of test compound that reduced the assembly or disassembly by 50% as compared to solvent controls.[37]

Assay for DNA Relaxation Activity of Topoisomerase I. The method described by Larsen et al.[38] was followed. Briefly, 18 μL of buffer A (50 mM Tris, pH 7.5, 60 mM KCl, 0.5 mM DTT, 0.5 mM EDTA), containing 200 ng of pBR322, and the amount of purified topoisomerase I prepared from calf thymus which resulted in 100% relaxation were added to an Eppendorf tube containing 2 μL of either vehicle alone or the test compound in vehicle. After 30 min incubation, the reaction mixture was analyzed on a 1% agarose gel and run at 35 mA overnight in Tris-borate-EDTA (90 mM Tris, 90 mM borate, 2 mM EDTA, pH 8.3) buffer. These so-called neutral gels permitted the detection of compounds which inhibit relaxation. Gels were stained with ethidium bromide, photographed under UV illumination, and the EC_{50} value, defined as the 50% efficacy concentration (i.e., the concentration at which 50% of the assays were positive for inhibition, which was generally the calculated intermediate value between the last active and the first active concentration calculated as the log mean value), were determined.

Assay for kDNA Decatenation Activity of Topoisomerase II. Briefly, 18 μL of buffer A (50 mM Tris, pH 7.5, 60 mM KCl, 0.5 mM DTT, 0.5 mM EDTA) containing 200 ng of kDNA and one unit of Drosophila topoisomerase II, identified as the amount of enzyme which resulted in the complete decatenation of 200 ng of kDNA, were added to an Eppendorf tube containing 2 μL of either vehicle (DMSO) alone or the test compound in DMSO. After 30 min incubation, the reaction mixture was analyzed on a 1% agarose gel and run at 35 mA overnight in Tris-borate-EDTA (89 mM Tris, 89 mM borate, 2 mM EDTA, pH 8.3) buffer. Gels were stained with ethidium bromide, photographed under UV illumination, and the EC_{50} value, defined as the 50% efficacy concentration, were calculated as described above for topoisomerase I inhibition.

Acknowledgment. We thank Dr. S. Cros (Toulouse, France) for a generous gift of murine P388 leukemia cell culture. Thanks are due also to Laboratoires Pierre Fabre for a fellowship (L. Daley). The authors are indebted to G. Flad (Inst. Curie) for performing the NMR spectra and to E. Chazottes, J. Astruc, J.-M. Barret, and A. Limouzy (P. Fabre) who realized the pharmacological experimentation.

Supporting Information Available: ^1H NMR data for all compounds which do not appear in the Experimental Section (3 pages). Ordering information is given on any current masthead page.

References

(1) (a) Jardine, I. *Podophyllotoxin in Anticancer Agents Based on Natural Products Models*; Academic Press: New York, 1980; pp 319−351. (b) Stähelin, H.; von Wartburg, A. From Podophyllotoxin Glucoside to Etoposide. *Prog. Drug. Res.* **1989**, *33*, 169−266. (c) Stähelin, H.; von Wartburg, A. The Chemical and Biological Route from Podophyllotoxin Glucoside to Etoposide: Ninth Cain Memorial Award Lecture. *Cancer Res.* **1991**, *51*, 5−15. (d) Sackett, D. L. Podophyllotoxin, Steganacin and Combretastatin, Natural Products that Bind at the Colchicine Site of Tubuline. *Pharmac. Ther.* **1993**, *59*, 163−228.

(2) Kelly, M. G.; Hartwell, J. L. The Biological Effects and the Chemical Composition of Podophyllotoxin. A review. *J. Natl. Cancer Inst.* **1954**, *14*, 967−1010.

(3) (a) Stahelin, L. VM 26, a new podophyllotoxin glucoside derivative with anti-L-1210 activity. *Proc. Am. Assoc. Cancer Res.* **1969**, *10*, 86−86. (b) Stähelin, H. 4′-Demethyl-epipodophyllotixin thenylidene glucoside (VM 26), a Podophyllum Compound with

a New Mechanism of Action. *Eur. J. Cancer* **1970**, *6*, 303−311. (c) Stähelin, H. Activity of a New Glycosidic Lignan Derivative (VP 16-213) Related to Podophyllotoxin in Experimental Tumors. *Eur. J. Cancer* **1973**, *9*, 215−221.

(4) Belani, C. P.; Doyle, L. A.; Aigner, J. Etoposide: Current Status and Future Perspectives in the Management of Malignant Neoplasms. *Cancer Chemother. Pharmacol.* **1994**, *34* (suppl.), S118−S126.

(5) Kobayashi, K.; Ratain, M. J. Pharmacodynamics and Long-Term Toxicity of Etoposide. *Cancer Chemother. Pharmacol.* **1994**, *34* (suppl.), S64−S68.

(6) (a) Wozniak, A. J.; Ross, W. E. DNA Damage as a Basis for 4′-Demethyl-epipodophyllotoxin-9-(4,6-*O*-ethylidene-*β*-D-glucopyranoside) (Etoposide) Cytotoxicity. *Cancer Res.* **1983**, *43*, 120−124. (b) Minocha, A.; Long, B. Inhibition of the DNA Catenation Activity of Type II Topoisomerase by VP-16−213 and VM-26. *Biochem. Biophys. Res. Commun.* **1984**, *122*, 165−170. (c) Burden, D. A.; Kingma, P. S.; Froelich-Ammon, S. J.; Bjornsti, M.-A.; Patchan, M. W.; Thompson, R. B.; Osheroff, N. Topoisomerase II Etoposide Interaction Direct the Formation of Drug-induced Enzyme-DNA Cleavage Complexes. *J. Biol. Chem.* **1996**, *46*, 29238−29244.

(7) (a) Saito, H.; Yoshikawa, H.; Nishimura, Y.; Kondo, S.; Takeuchi, T.; Umezawa, H. Studies on Lignan Lactone Antitumor Agents. I. Synthesis of Aminoglycosidic Lignan Variants Related to Podophyllotoxin. *Chem. Pharm. Bull.* **1986**, *34*, 3733−3740. (b) Saito, H.; Yoshikawa, H.; Nishimura, Y.; Kondo, S.; Takeuchi, T.; Umezawa, H. Studies on Lignan Lactone Antitumor Agents. II. Synthesis of N-alkylamino- and 2,6-dideoxy-2-aminoglycosidic Lignan Variants Related to Podophyllotoxin. *Chem. Pharm. Bull.* **1986**, *34*, 3741−3746.

(8) (a) Showalter, H. D. H.; Winters, R. T.; Sercel, A. D.; Michel, A. Facile Synthesis of Thioglucose Analogs of the Anticancer Agent, Etoposide. *Tetrahedron Lett.* **1991**, *32*, 2849−2852. (b) Allevi, P.; Anastasia, M.; Ciuffreda, P. A Short and Simple Synthesis of the Thioglucose Analogs of the Antitumor Agent, Etoposide. *Tetrahedron Lett.* **1991**, *32*, 6227−6230. (c) Saito, H.; Nishimura, Y.; Kondo, S.; Umezawa, H. Synthesis of the All Four Possible Diastereoisomers of Etoposide and its Aminoglycosidic Analogues via Optical Resolution of (±)Podophyllotoxin by Glycosidation with D- and L-sugars. *Chem. Lett.* **1987**, 799−802.

(9) Allevi, P.; Anastasia, M.; Ciuffreda, P. The First Synthesis of the N-glucosyl Analogues of the Antitumor Agent, Etoposide. *Tetrahedron Lett.* **1993**, *34*, 7313−7316.

(10) Allevi, P.; Anastasia, M.; Ciuffreda, P.; Scala, A. A Simple Synthesis of C-Glucosides Related to the Antitumor Agent Etoposide. *J. Carbohydr. Chem.* **1993**, *12*, 209−222.

(11) (a) Kudoh, S.; Fokuoka, M.; Furuse, K.; et al. Phase I Clinical Trial and Pharmacokinetic Study of the New Etoposide Analog, NK 611 in Patients with Advanced Solid Tumors. *Ann. Oncol.* **1993**, *3* (suppl. 1), 146−146. (b) Hanauske, A.-R.; Wüster, K. C.; Lehmer, A.; Rotter, M.; Schneider, P.; Kaeser-Fröhlich, A.; Rastetter, J.; Depenbrock, H. Activity of NK 611, a New Epipodophyllotoxin Derivative, Against Colony Forming Units from Freshly Explanted Human Tumours In Vitro. *Eur. J. Cancer* **1995**, *31A*, 1677−1681.

(12) (a) Hauser, F. M.; Ellenberger, S. Syntheses of 2,3,6-Trideoxy-3-amino- and 2,3,6-Trideoxy-3-nitrohexoses. *Chem. Rev.* **1986**, *86*, 35−67. (b) Pelyvas, I. F.; Monneret, C.; Herczegh, P. *Synthetic Aspects of Aminodeoxy Sugars of Antibiotics*; Springer-Verlag: Berlin, Heidelberg, 1988.

(13) Arcamone, F. Doxorubicin Anticancer Antibiotics. *Medicinal Chemistry*; Academic Press: New York, 1981; Vol. 17.

(14) Sztaricskai, F.; Bognár, R. *The Chemistry of the Vancomycin Group of Antibiotics*; Akadémiai Kiadó: Budapest, 1984; pp 91−201.

(15) Roger, P.; Monneret, C.; Fournier, J.-P.; Choay, P.; Gagnet, R.; Gosse, C.; Letourneux, Y.; Atassi, G.; Gouyette, A. Rationale for the Synthesis and Preliminary Biological Evaluation of Highly Active New Antitumor Nitrosoureido Sugars. *J. Med. Chem.* **1989**, *32*, 16−23.

(16) (a) Atassi, G.; Dumont, P.; Gosse, C.; Fournier, J.-P.; Gouyette, A.; Roger, P. Characterization of the Anti-Tumour Activity Against Solid Tumours of a New Nitrosoureido Sugar: CY 233. *Cancer Chemother. Pharmacol.* **1989**, *25*, 205−209. (b) Dumont, P.; Atassi, G.; Roger, P. Evaluation of a New Nitrosoureido Water-Soluble Sugar, Ecomustine or CY 233 (NSC 609224), on Human Xenografts. *In Vivo* **1990**, *4*, 61−64.

(17) Florent, J. C.; Monneret, C. Stereocontrolled route to 3-amino-2,3,6-trideoxy-hexopyranoside. K10 Montmorillonite as a glycosidation reagent for Acosaminide Synthesis. *J. Chem. Soc., Chem. Commun.* **1987**, 1171−1172.

(18) Daley, L.; Roger, P.; Monneret, C. Synthesis of 6-hydroxy-L-daunosamine and L-daunosamine derivatives. *J. Carbohydr. Chem.* **1997**, *16*, 25−48.

(19) (a) Klotz, W.; Schmidt, R. R. Anomeric *O*-alkylation of *O*-acetyl-Protected Sugars. *J. Carbohydr. Res.* **1994**, *13*, 1093−1101. (b) Schmidt, R. R.; Michel, J. Direct *O*-glycosyl Trichloracetamidate Formation. Nucleophilicity of the Anomeric Oxygen Atom. *Tetrahedron Lett.* **1984**, *25*, 821−824.

(20) Priebe, W.; Grynkiewicz, G.; Neamati, N. 2-Deoxy-1-*O*-silylated-*β*-hexopyranose, Useful Glycosyl Donors and Synthetic Intermediates. *Tetrahedron Lett.* **1991**, *32*, 2079−2082.

(21) Kolar, C.; Kneissl, G.; Knödler, U.; Dehmel, K. Semisynthetic Rhodomycins: Novel Glycosylation Methods for the Synthesis of Anthracycline Oligosaccharides. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 809−810.

(22) Kolar, C.; Moldenhauer, H.; Kneissl, G. A New Approach to the Synthesis of Etoposide. *J. Carbohydr. Chem.* **1990**, *9*, 571−583.

(23) (a) Kuhn, M.; Keller-Juslén, C.; von Wartburg, A. Partial synthesis of 4′-Demethylepidodophyllotoxin. *Helv. Chim. Acta* **1969**, *52*, 944−947. (b) Kuhn, M.; von Wartburg, A. Mitosis-inhibiting Substances. Glycosylation Process. II. Glycosides of 4′-Demethylepipodophyllotoxin. *Helv. Chim. Acta* **1969**, *52*, 948−955.

(24) (a) Lee, K.-H.; Imakura, Y.; Haruna, M.; Beers, S. A.; Thurston, L. S.; Dai, H.-J.; Chen, C.-H. Antitumor Agents. 107. New Cytotoxic 4′-alkylamino analogues of 4′-Demethyl-Epipodophyllotoxin as Inhibitors of Human DNA Topoisomerase II. *J. Nat. Prod.* **1989**, *52*, 606−611. (b) Thurston, L. S.; Imakura, Y.; Haruna, M.; Li, D.-H.; Liu, Z.-C.; Liu, S.-Y.; Cheng, Y.-C.; Lee, K. H. Antitumors Agents. 100. Inhibition of DNA Topoisomerase II by Cytotoxic Etherane Ester Derivatives of Podophyllotoxin and α-Peltatin. *J. Med. Chem.* **1989**, *32*, 604−608.

(25) Vickery, E. H.; Pahler, L. F.; Eisenbraun, E. J. Selective *O*-Demethylation of Catechol Ethers. Comparison of Boron Tribromide and Iodotrimethylsilane. *J. Org. Chem.* **1979**, *44*, 4444−4446.

(26) Jung, M. E.; Lyster, M. A. Quantitative Dealkylation of Alkyl Ethers via Treatment with Trimethylsilyl Iodide. A New Method for Ethers Hydrolysis. *J. Org. Chem.* **1977**, *42*, 3761−3764.

(27) Olah, G. A.; Narang, S. C.; Gupta, B. G. B.; Malhotra, R. Synthetic Methods and Reactions. 62. Transformations with Chlorotrimethylsilane/Sodium Iodide, a Convenient in Situ Iodotrimethylsilane Reagent. *J. Org. Chem.* **1979**, *44*, 1247−1251.

(28) Morita, T.; Yoshida S.; Okamoto Y.; Sakurai H. Chlorotrimethylsilane/Sodium Iodide: A New Reagent for Conversion of Alcohols into Iodides. *Synthesis* **1979**, 379−379.

(29) Groutas, W. C.; Felker D. Synthetic Application of Cyanotrimethylsilane, Iodotrimethylsilane, Azidotrimethylsilane and Methylthiomethyltrimethylsilane. *Synthesis* **1980**, 861−868.

(30) Klein, L. L.; Yeung, C. M.; Chu, D. T.; McDonald, G. J.; Clement, J. J.; Plattner, J. J. Synthesis and Antitumor Activity of Structural Analogues of the Epipodophyllotoxins. *J. Med. Chem.* **1991**, *34*, 984−992.

(31) This demethylation−epimerization tandem reaction has been recently extended to the synthesis of 4-(4-fluoroanilino) epipodophyllotoxin (or NPF), a potent antitumor compound,[32] by further treatment with 4-fluoroaniline instead of barium carbonate; see Daley, L.; Meresse, P.; Bertounesque, E.; Monneret, C. A one-pot Efficient Synthesis of the Potent Cytotoxic Podophyllotoxin Derivative, NPF. *Tetrahedron Lett.* **1997**, *38*, 2673−2676.

(32) Lee, K.-H.; Beers, S. A.; Mori, M.; Wang, Z.-Q.; Kuo, Y.-H.; Li, L.; Liu, S. Y.; Chang, J.-Y.; Han, F. S.; Cheng, Y.-C.; Antitumor Agents. 111. New 4-Hydroxylated and 4-Halogenated Anilino Derivatives of 4′-Demethylepipodophyllotoxin as Potent Inhibitors of Human DNA Topoisomerase. II. *J. Med. Chem.* **1990**, *33*, 1364−1368.

(33) Kuhn, M.; von Warburg, A. Mitosis-inhibiting Substances. XX. Cleavage of Acyl Protective Groups in Alkali-sensitive Glucosides. *Helv. Chim. Acta* **1968**, *51*, 163−168.

(34) Long, B. H.; Casazza, A. M. Structure−activity relationships of VP-16 analogues. *Cancer Chemother. Pharmacol.* **1984**, *34*, 526−531.

(35) Gaskin, F.; Babtor, C. R. Turbidimetric Studies of the In Vitro Assembly and Disassembly of Porcine Neurotubules. *J. Mol. Biol.* **1974**, *89*, 737−758.

(36) Bai, R.; Pettit, G. R.; Hamel, E. Dostatin 10, a powerful cytostatic peptide derived from a marine animal. *Biochem. Pharmacol.* **1993**, *39*, 13560−13565.

(37) Shelansky, M. L.; Gaskin, F.; Cantor, C. R. Microtubule Assembly in the Absence of Added Nucleophiles. *Proc. Natl. Acad. Sci.* **1973**, *70*, 765−786.

(38) Larsen, A. K.; Grondard, L.; Couprie, J.; Desoize, B.; Comoe, L.; Jardillier, J.-C.; Riou, J.-F. The antileukaemic alkaloid fagaronine is an inhibitor of DNA topoisomerases I and II. *Biochem. Pharmacol.* **1993**, *46*, 1403−1412.

JM9800752